Behavioural mechanisms underlying infant care in male and female Mongolian gerbils.

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BSc (Hons)

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Department of Molecular and Life Sciences,

Dundee College of Technology

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"Whatever you do, work at it with all your heart, as though you were working for the Lord and not for men."

Colossians Ch 3, verse 23.

"I give thanks to Christ Jesus our Lord, who has given me strength for my work. To the eternal King, immortal and invisible, the only God - to Him be honour and glory."

1 Timothy Ch 1, verses 12 and 17.

ELSPETH C. HENRY. BEHAVIOURAL MECHANISMS UNDERLYING INFANT CARE IN MALE AND FEMALE MONGOLIAN GERBILS.

The induction of parental behaviour in naive animals has been studied in several species. Male and female adult Mongolian gerbils, Meriones unguiculatus were singly exposed to protected pups (PP exposures). Exposures lasted for ten minutes, and were carried out each day. Behaviour patterns shown by the adults were recorded, and their frequencies measured. After several PP exposures (3 - 18), adults were exposed to unprotected pups (UP exposures) in order to detect whether or not the normal aggressive response to pups was still present, or had been overcome and replaced by parental responsiveness. Preliminary experiments showed the aggressive response could be overcome in as little as five ten minute exposures. A variety of parental behaviour patterns were shown by some individuals, suggesting that stages may exist in the process under investigation. Following on from preliminary experiments, the effect of increasing the number of both PP and UP exposures was investigated. Increasing PP and UP exposures increased the percentage of animals responding non-aggressively towards pups. However no increase was seen in the range of parental responses shown. Again, results suggested the development of the parental response was a non-unitary process occurring in stages: first the overcoming of fear of pups or aggression towards pups; second, investigation of the pup; third, the development of parental responsiveness.

The role of olfactory and auditory cues from the pups were next investigated. If a pup bore the scent gland sebum of the experimental adult, aggression was overcome more quickly than before. Also, more parental behavior patterns were shown. If the pup bore the experimental adult's urine, aggression was overcome more quickly than in preliminary experiments, but not as quickly as when the pups bore the adult's sebum.

No correlation was found between the rate of ultrasonic calling and the rate of the induction of parental responsiveness. This was thought to be an artefact of the recording procedure, since the source of individual calls was not identified, and the frequency of calls could therefore have been increased due to adults calling.

Parental responsiveness appeared to be maintained 2 weeks after its induction, but not 10 weeks after induction. An exception to this was the animals exposed to pups smeared with the experimental adults' sebum, who did not appear to maintain responsiveness even up to 2 weeks after induction.

Overall twice as many males as females were able to be induced to show parental responsiveness. Males overcame their aggression to pups, and showed parental responsiveness more quickly than females did.

Further work arising from the present studies would include a more detailed study of both the influence of ultrasonic calling by pups on the development of parental responsiveness and the quicker development of parental responsiveness found when pups bore an odour familiar to the adults.

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CONTENTS PAGE

Abstract 1

Acknowledgements 2

Contents 4

List of figures and tables 11

CHAPTER ONE GENERAL INTRODUCTION 17

1.1 Parental behaviour 17

1.1 i Parental behaviour in juveniles 19

1.2 Priming 21

1.2 i Parental or non-parental? 21

1.2 ii Maternal induction 22

1.2 iii Paternal induction 23

1.3 Mechanisms producing parental behaviour 24

1.3 i Hormonal mechanisms 24

1.3 ii Non-hormonal mechanisms 26

1.4 Infanticide 27

1.5 Previous use of methods employed in current work 28

1.5 i Use of enclosed pups 28

1.5 ii Continuous exposure method 30

1.5 iii Stimuli for priming 30

1.6 The Mongolian gerbil 33

1.7 Aims of the study 34

CHAPTER TWO GENERAL METHODS 35

2.1 Sources of Animals 35

2.1 i Experimental Animals 35

2.1 ii Stimulus Animals 38

2.1 iii Maintenance 38

2.1 iv Surgical Technique - Bilateral vasectomy 39

2.2 Materials 40

2.2 i Observation Chamber 40

2.2 ii Stimulus Pup's Protective Cage 40

2.3 General Behavioural Testing Procedure 42

2.3 i Removal of Pups from the Parental Nest 45

2.4 Recording of Results 45

2.4 i Checksheets 45

2.4 ii Ultrasonics Checksheets 46

2.5 Analysis of Results 46

CHAPTER THREE DESCRIPTIONS OF BEHAVIOUR PATTERNS 48

3.1 Behaviour Patterns 48

3.2 Ultrasonic Vocalizations 51

CHAPTER FOUR PRODUCTION OF PARENTAL RESPONSIVENESS IN NAIVE MALE AND FEMALE MONGOLIAN GERBILS 52

4.1 Introduction 52

4.2 Method 52

4.2 i Analysis of results 53

4.3 Results 54

4.3 i Comparison of PP with C exposures 54

4.3 i a Frequencies of behaviour patterns 54

4.3 i b Sex differences (PP and C exposures) 54

4.3 ii UP exposures 57

SUMMARY 57

4.4 Discussion 60

4.4 i Comparison of PP with C exposures 60

4.4 ii UP exposures 61

CHAPTER FIVE DEVELOPMENT OF PARENTAL RESPONSIVENESS IN NAIVE MALE AND FEMALE MONGOLIAN GERBILS. 64

5.1 Introduction 64

5.2 Method 64

5.2 i Analysis of results 65

5.3 Results 67

5.3 i PP Exposures 67

5.3 i a First 5 PP exposures 67

5.3 i b All (18) PP exposures 67

5.3 i c Comparison of first 5 with all (18) PP exposures 67

5.3 ii Latency to a non-aggressive response 70

5.3 iii Intermediate UP Exposures 74

5.3 iii a Frequencies of patterns 74

5.3 iv Final UP exposures 74

5.3 iv a Frequencies of patterns 74

5.3 v Comparison of preliminary and current experiments 75

5.3 v a Latency to a non-aggressive response 75

5.3 v b Comparison of first 5 PP exposures 80

5.3 v c Comparison of final UP exposures 80

SUMMARY 80

5.4 Discussion 85

5.4 i PP Exposures 85

5.4 i a First five 85

5.4 i b Comparison of first five with all PP exposures 86

5.4 ii Latency to a non-aggressive response 87

5.4 iii UP exposures 88

5.4 iv Comparison of preliminary with current experiments 90

5.4 iv a Comparison of latency to a non-aggressive response 90

5.4 iv b Comparison of first five PP exposures 91

5.4 iv c Comparison of behaviour frequencies in UP exposures 92

CHAPTER SIX PUP STIMULI I - ODOUR. 93

6.1 Introduction 93

6.2 Method 96

6.3 Results 99

6.3 i Sebum results 99

6.3 i a UP Exposures 99

6.3 i b Test compared with control 105

6.3 ii Urine results 105

6.3 iia UP exposures (males compared with females) 105

6.3 iib Test compared with control 109

6.3 iii Comparison of 'sebum' with 'urine' experiments 114

SUMMARY 117

6.4 Discussion 117

6.4 i Sebum experiment 117

6.4 ii Urine experiment 121

6.4 iii Sebum/urine 122

CHAPTER SEVEN PUP STIMULI II - ULTRASONIC VOCALIZATIONS. 125

7.1 Introduction 125

7.2 Method 126

7.3 Results 128

7.3 i Correlation of rate of calls with adult responses 128

7.3 ii Sonagrams 130

SUMMARY 134

7.4 Discussion 134

7.4 i Correlation of rate of calls with adult responses 134

7.4 ii Sonagrams 138

CHAPTER EIGHT MAINTENANCE OF THE DEVELOPED PARENTAL RESPONSIVENESS 139

8.1 Introduction 139

8.2 Method 140

8.2 i Maintenance periods 142

8.2 ii Group 1 142

8.2 iii Group 2 142

8.2 iv Group 3 143

8.3 Results 143

8.3 i Aggression 143

8.3 ii Parental patterns 146

8.3 iii Sex differences 162

SUMMARY 162

8.4 Discussion 166

8.4 i Aggression 166

8.4 ii Parental patterns 168

8.4 iii Sex differences 171

CHAPTER NINE GENERAL DISCUSSION AND CONCLUSIONS 175

REFERENCES 183

LIST OF FIGURES AND TABLES PAGE

CHAPTER ONE

Fig. 1.1 Litter of one day old pups with their father. 31

Fig. 1.2 Litter of seven day old pups with their father. 31

Fig. 1.3 Litter of fourteen day old pups with their mother. 32

CHAPTER TWO

Fig. 2.1 Numbers of litters born each month between December 1983 and December 1985. 36

Fig. 2.2 Litter sizes. 37

Fig. 2.3 Observation chamber. 41

Fig. 2.4 Stimulus pup's protective cage. 41

Fig. 2.5 Checksheet for recording behaviour patterns. 43

Fig. 2.6 Covering of cage top during experiments. 44

Fig. 2.7 Checksheet for recording ultrasonic vocalizations. 47

CHAPTER FOUR

Tab. 4.1 Comparison of frequencies of behaviour patterns shown by males in PP and C exposures. 55

Tab. 4.2 Comparison of frequencies of behaviour patterns shown by females in PP and C exposures. 56

Tab. 4.3 Frequencies of parental patterns shown by animals showing a non-aggressive response in UP exposure. 58

Tab. 4.4 Percentages of animals still showing an aggressive 59

response to an unprotected pup after a total of 5 PP and 4 C exposures.

CHAPTER FIVE

Tab. 5.1 Comparison of frequencies of behaviour patterns shown by males and females in first five PP exposures. 68

Tab. 5.2 Comparison of frequencies of behaviour patterns shown by males and females over all PP exposures. 69

Tab. 5.3 Comparison of frequencies of behaviour patterns shown by males between first five and all PP exposures. 71

Tab. 5.4 Comparison of frequencies of behaviour patterns shown by females between first five and all PP exposures. 72

Tab. 5.5 Latency to first non-aggressive response shown. 73

Tab. 5.6 Numbers of animals showing a non-aggressive response in the final three UP exposures. 76

Tab. 5.7 Comparison of frequencies of behaviour patterns shown by males and females in the final UP exposures. 77

Tab. 5.8 Numbers of animals showing aggressive and non-aggressive responses in UP exposures (preliminary experiment). 78

Tab. 5.9 Numbers of animals showing aggressive and non-aggressive responses in UP exposures (current experiment). 78

Tab. 5.10 Percentages of animals showing a non-aggressive 79

response after 5, 10, 15 and 18 PP exposures, and after 18 PP + 1 UP exposures in preliminary and

Tab. 5.11 Comparison of frequencies of behaviour patterns shown by males in first five PP exposures between preliminary and current experiments. 81

Tab. 5.12 Comparison of frequencies of behaviour patterns shown by females in first five PP exposures between preliminary and current experiments. 82

Tab. 5.13 Comparison of frequencies of behaviour patterns shown by males in UP exposures between preliminary and current experiments. 83

Tab. 5.14 Comparison of frequencies of behaviour patterns shown by females in UP exposures between preliminary and current experiments. 84

CHAPTER SIX

Fig. 6.1 Sequence of exposures in 'sebum' and 'urine' experiments. 98

Tab. 6.1 Comparison of frequencies of behaviour patterns shown by males and females over first five 'sebum' UP exposures. 100

Tab. 6.2 Comparison of frequencies of behaviour patterns shown by males and females over all (10) 'sebum' UP exposures 101

Tab. 6.3 Comparison of frequencies of behaviour patterns shown by males over all UP exposures with first three UP exposures in 'sebum' experiment. 102

Tab. 6.4 Comparison of frequencies of behaviour patterns shown by females over all UP exposures with first three UP exposures in 'sebum' experiment. 103

Tab. 6.5 Comparison of numbers of PP exposures required to produce a non-aggressive response to an unprotected pup in 'sebum' experiment. 104

Tab. 6.6 Comparison of frequencies of behaviour patterns shown by males in first three (sebum) UP exposures and control UP exposures. 106

Tab. 6.7 Comparison of frequencies of behaviour patterns shown by females in first three (sebum) UP exposures and control UP exposures. 107

Tab. 6.8 Comparison of numbers of PP exposures needed to produce a non-aggressive response to an unprotected pup in sebum and control experiments. 108

Tab. 6.9 Comparison of frequencies of behaviour patterns shown by males and females in 'urine' UP exposures. 110

Tab. 6.10 Comparison of numbers of PP exposures needed to produce a non-aggressive response to an unprotected pup in 'urine' experiment. 111

Tab. 6.11 Comparison of frequencies of behaviour patterns shown by males in 'urine' UP exposures and controls. 112

Tab. 6.12 Comparison of frequencies of behaviour patterns shown by females in 'urine' UPexposures and controls. 113

Tab. 6.13 Comparison of numbers of PP exposures required to produce a non-aggressive response to an unprotected pup in 'urine' and control experiments. 115

Tab. 6.14 Comparison of numbers of PP exposures needed to produce a non-aggressive response to an unprotected pup in 'sebum' and 'urine' experiments. 116

CHAPTER SEVEN

Fig. 7.1 Recording ultrasonic vocalizations. 127

Fig. 7.2 Playback of ultrasonic vocliztons. 129

Tab. 7.1 Median values of frequencies of parental behaviours and rate of ultrasonic calling. 131

Tab. 7.2 Median rate of calls per minute in PP exposures and numbers of PP exposures required to produce a non-aggressive response in UP exposures. 132

Fig. 7.3 - 7.5 Sonagrams 133

Fig. 7.6 - 7.8 Sonagrams 135

CHAPTER EIGHT

Tab. 8.1 Testing regime for maintenance experiments 141

Tab. 8.2 Numbers of animals showing an aggressive on at least one occassion in a series of UP exposures 144

Tab. 8.3 Numbers of aggressive responses during series of UP exposures 145

Figs.8.1 - 8.14 Frequencies of behaviour patterns shown during maintenance UP exposures. 147

Fig. 8.1 Inactive. 147

Fig. 8.2 Groom self. 148

Fig. 8.3 Scratch cage. 149

Fig. 8.4 Sniff own cage. 150

Fig. 8.5 Sniff pup. 151

Fig. 8.6 Tear paper. 152

Fig. 8.7 Burrow. 153

Fig. 8.8 Sit over. 154

Fig. 8.9 Gnaw own cage. 155

Fig. 8.10 Bury pup. 156

Fig. 8.11 Sit over and nest build. 157

Fig. 8.12 Retrieve. 158

Fig. 8.13 Gather. 159

Fig. 8.14 Total time near ($\leq$ 2.5cm) pup. 160

161

Tab. 8.4 Comparison of frequencies of behaviour patterns shown by Group 2 males and females at 2wk M UP exposures. 163

Tab. 8.5 Comparison of frequencies of behaviour patterns shown by Group 3 males and females at 2wk M UP exposures. 164

Tab. 8.6 Comparison of frequencies of behaviour patterns shown by Group 3 males and females at 10wk M UP exposures. 165

CHAPTER ONE GENERAL INTRODUCTION.

1.1 Parental Behaviour

Parental behaviour is the term used to describe the behaviour patterns shown by parent organisms which can be seen to contribute to the well-being and survival of young. The term can cover behaviour patterns shown before young are born, for example, several animal species prepare a nest area for the arrival of their young. Parental behaviour continues for various lengths of time. The two extremes are shown by birds, with altricial birds (for example the thrush (*Turdus* spp.) hatching out at an immature stage of development, and being entirely dependent on one or both parents for feeding. On the other hand, precocial birds (for example the duck (*Anas platyrhynches*) are well developed and leave the nest on hatching. Parental behaviour is seen in several phyla, including Annelids, for example the leech *Hellobdella striata* which carry their young by attachment to their own bodies through the developmental stages of egg, larva and juvenile. At the juvenile stage, food is given to the young for a further three weeks, allowing growth and providing protection (Kutschera and Wirtz, 1987). Parental behaviour is also seen in several classes of chordates including birds, the majority of the mammals, and in some amphibians for example *Eleutherodactylus coqui*, a tropical frog which provides both maternal and paternal care for the eggs throughout embryonic development (Townsend and Moger, 1987). Parental behaviour has been studied in great detail in primates. Both male and female adults as well as sub-adult animals of several species are known to 'help' in infant care

(Swartz and Rosenblum, 1981). Behaviour patterns not strictly related directly to providing care for young may also be included in the term parental behaviour, for example territory defence and courtship feeding, which if not performed (in the relevant species) would almost certainly reduce or totally negate reproductive success.

The provision of parental care will incur a cost to the animals involved. However the benefit arising as a result of this investment is the increased likelihood of the survival of the young, and the preservation of the individual's genotype. This has been described as parental investment (Trivers, 1972, cited in Kurland and Gaulin, 1984), and defined as "any investment by the parent in an individual offspring that increases the individual's chances of surviving (and hence reproductive success) at the cost of the parent's ability to invest in other offspring."

Both male and female animals are involved in parental behaviour, either together or singly, with variations being seen between species. Maternal behaviour is generally taken as being applied to parental activities shown by the female parent, whereas paternal behaviour refers to parental activities shown by the male parent. It has been suggested that the likelihood of paternal investment in the rearing of the young increases when the animals in question are monogamous, that is when a pair join for breeding and stay together for several breeding seasons, but not necessarily for a lifetime (Kleiman, 1974). The species used in the present study, the Mongolian gerbil, Meriones unguiculatus, is known to

naturally live in extended family groups, that is a monogamous pair with young from two or more litters (Elwood, 1983). This living habit could therefore account for the development of paternal behaviour which is known to be shown by male Mongolian gerbils (Elwood, 1975). The majority of authors support the idea of the male's role in the raising of gerbil pups, although the presence of the male was once implicated in causing the female to neglect the pups, resulting in high levels of pup mortality (Ahroon and Fidura, 1976). This was later explained (Elwood and Broom, 1978) as likely to be due to either the transport by man of the females when they were pregnant, or inappropriate lighting and housing conditions used during the study producing high temperatures in the observation cages (Gerling and Yahr, 1979).The results were later accepted as unusual by one of the authors (Klippel, 1979, nee Ahroon), and were most likely due to the transit of the animals. The responses of male and female Mongolian gerbils towards pups are considered to be more or less equivalent, with the obvious exception of lactation (Elwood, 1975).

1.1 i Parental behaviour in juveniles.

Parental behaviour has been shown to occur in juvenile animals (that is members of a litter not yet mature) prior to the development of infanticide in mice (Gandelman et al, 1970), hamsters (Rowell, 1961a) and gerbils (Elwood, 1980). The development of the killing of newborn pups occurs in mice when pups are approximately 32 days old, and in gerbils when the pups are between 30 and 60 days old. It has been suggested that in gerbils

the occurrence of the development of pup killing at this age facilitates the protection of offspring born as a result of mating which is known to occur if a female is lactating (Norris and Adams, 1971). This would mean newborn pups in a family group and 30 - 40 day old young living in the same group would likely be full-sibs. The inhibition of pup killing by these juveniles would therefore help the survival of their family group.

A study of the role of juvenile gerbils raised in colonies in semi-natural conditions (Ostermeyer and Elwood, 1984) has shown that several parental behaviours are shown towards the new pups of a subsequent litter by the juveniles. As a consequence of this, parents show less self-grooming and licking of pups. It is likely that new pups benefit from increased pup care due to the presence of the juveniles in the nest as with the presence of the father (Elwood and Broom, 1978). In contrast, experiments carried out in cages suggest that new born pups may suffer retarded growth possibly as a result of juveniles taking milk from the mother, when juveniles remain present with the family group (Ostermeyer and Elwood, 1984). Possibly in the burrow situation the female would exclude the juveniles from the maternal nest. It is perhaps advantageous to the juveniles to stay with the parents, and help with raising the next litter. Experience of parental behaviour may be gained by juveniles at this stage.

1.2 Priming

Parental behaviour in rodents is not shown exclusively by parent animals. The development of parental behaviour in non-parent adult animals by continuous exposure to pups in the adult's cage has been studied in several species. Pups are placed in the adult's cage, and the adult's behaviour is then recorded and parental responses measured (see for example Rosenblatt, 1967). Several terms have been used to describe the induction of this behaviour including priming (Hinde, 1966), and sensitization or concaveation (Noirot, 1972b). Priming has been shown in male and female adult animals. The response is non-unitary, that is, has no one causal factor (Hinde, 1959). Several aspects of priming or behavioural induction are discussed throughout the current chapter.

1.2 i Parental or non-parental?

A difference between the method used in this work and in several earlier pieces of work is in the decision of whether or not an animal's response to a pup is parental. An adult showing retrieval of pups to the nest has often been considered as an indication that the adult is parental. However, Plume *et al* (1968) dispute this, pointing out that behaviours cannot be regarded as parental unless they are directed towards young (as distinct from a response produced in an animal which is later performed when in the presence of a pup). Grota and Ader (1969) used 'time spent in nest' as a reflection of maternal behaviour in rats. Parental behaviour is a set of stereotyped patterns. Each one may have developed

(partially) independently, therefore it is "unscientific" to say one pattern alone equals parental behaviour. It is preferable to base the decision as to whether or not an animal is parental on the range of parental patterns it has shown, rather than on one or two alone. For this reason no one pattern alone is here taken as being indicative of a parental response.

1.2 ii Maternal induction

Induced maternal behaviour has been studied and described for several rodent species. There are several differences in the responses of naive females to pups between species. Rats initially tend to avoid pups (Wiesner and Sheard, 1933), whereas mice rapidly show maternal responses (Beniest-Noirot, 1958). Naive hamsters frequently attack pups on their first encounter (Richards, 1966) as do naive Mongolian gerbils (Elwood, 1977). The aggressive response shown by hamsters has been suggested as being due to the relatively short gestation period of 16 days (Noirot, 1972b), which results in 'younger' pups in terms of *in utero* development being born. These 'younger' pups do not inhibit aggression (presumably this is normally inhibited at parturition). This idea came about since 6 - 10 day old pups were less likely to produce an aggressive response in naive adults. Since gerbils do not have such a short gestation period as the hamster, the same reason for the same initial reaction to pups is unlikely. Another explanation is that the effect is due to the solitary lifestyle of hamsters, which causes hamsters to defend their territory against strangers, except during periods of mating. The same explanation cannot be applied to gerbils because they do not show a solitary lifestyle.

1.2 iii Paternal induction

Paternal behaviour has been increasingly studied in recent years. Less literature is available than that for maternal behaviour, although primates and rats have been studied extensively. The male parental behaviour in one marmoset species is thought to be hormonally controlled (Dixson and George, 1982). Brown (1986b), found male Long-Evans rats showed parental behaviour after exposure to pups. However longer exposure periods to pups were needed than with females, but despite this there were fewer components of parental behaviour than shown by females. Also parental behaviour was shown by fewer males than females. Several social factors of the rats' rearing as well as variations in pup stimuli are thought to be responsible for the differences between males and females. For both rats and mice it was found that males housed with lactating females showed little parental behaviour, but those housed alone showed a full range (Brown, 1986b; Gandelman et al, 1970). Infanticide is inhibited in male rats, mice and gerbils when they are housed with a pregnant female (Brown, 1986a; Elwood, 1977, 1980; Elwood and Ostermeyer, 1984a). The mechanism by which co-habitation with a pregnant female inhibits infanticide in rodents is unknown, but it has been suggested that maternal aggression causes the subordination of the male, thus inhibiting infanticide (Elwood and Ostermeyer, 1984c). In addition to this, male gerbils having raised a litter will never show infanticide again (Elwood, 1977). A review of the evolution of paternal investment in rearing young is provided by Kurland and Gaulin (1984).

Work on the production of parental behaviour by exposure to pups, which had successfully been carried out with rats, mice and hamsters, had never been achieved with gerbils due to their infanticidal tendencies. The initial aim of this work therefore was to see if indeed full parental behaviour could be produced in naive adults by exposure to pups.

1.3 Mechanisms producing parental behaviour

Two fields of thought exist with regard to the mechanism by which parental behaviours arise in individual naive animals.

1.3 i Hormonal mechanisms

In the natural situation of a rodent pair raising a litter, it is assumed that the mother is prepared for providing maternal care by changes in circulating hormones due to pregnancy. It is thought that the hormonal state of the animal is critical for the development of matenal behaviour. Several authors have tried to mimic the balance of hormones during pregnancy and parturition, but most of the earlier work has since been considered unreliable (see Noirot, 1972b for a review). Moltz et al (1970) significantly reduced the latency for naive female rats to respond maternally to pups by injecting them with estradiol benzoate, progesterone and prolactin in combination over 11 days. Other work has shown that

animals going through pregnancy but not parturition will act maternally if their young are given to them after caesarean section (Bridges, 1977). In this work Bridges concluded that the state of pregnancy and mother - young interactions were required for long term maintenance of maternal responsiveness. Terkel and Rosenblatt (1971, 1972) developed cross transfusion of blood between two rats such that the blood of a female just prior to or after parturition, or a pup-induced maternal female was passed to a naive female. Blood from newly parturient mothers was successful in inducing maternal behaviour in naive females (1972). Latency to the appearance of maternal behaviour was also reduced by the cross-transfusion of the blood of a very new mother, ie. up to 24 hours post partum. The effect was not seen using blood from a female 24 hours prior to parturition or 24 hours post parturition, implying a rapid hormonal change produces maternal behaviour which is then maintained possibly by pup stimulation. Blood from pup-induced maternal females was unable to reduce latencies to maternal behaviour (1971). This was taken as support for a non-hormonal basis for induced maternal behaviour.

The role of pituitary produced hormones in the display of parental behaviour has also been studied in detail. Oxytocin is thought to be involved in parturition (Fuchs, 1983), and this has led to investigations into its role in the induction of parental behaviour. Results overall suggest oxytocin acting in conjunction with oestrogen, either injected or endogenous, triggers a rapid (within minutes of pup exposure) onset of the display of maternal behaviour in virgin female rats (Pedersen, Ascher, Monroe and

Prange, 1982; Fahrbach, Morrell and Pfaff, 1984). Results of one experiment led to the conclusion that results could be variable, dependent on the breeding stocks of the strain of rat used, the level of oestrogen priming (when experimenting with ovariectomized females), and even the source of the oxytocin used (Rubin, Menniti and Bridges, 1983). Similar results were found by Ascher et al (Ascher, Pedersen, Hernandez and Prange, 1982). More recent work has found pre-test cage habituation affects the induction of maternal behaviour, with a two hour habituation shortening the latency to the display of maternal behaviour. Oddly this effect was not shown by either a one week habituation, or as expected a zero hour habituation (Fahrbach, Morrell and Pfaff, 1986). It has also been demonstrated that prolactin in combination with estradiol and progesterone has a role in priming a female rat to respond maternally to young at parturition (Bridges, DiBiase, Loundes and Doherty, 1985).

1.3 ii Non-hormonal mechanisms

Several other authors support the idea that maternal behaviour can be non-hormonally produced. The use of ovariectomized and hypophysectomized female rats, and castrated males, which could all be induced to show maternal behaviour by exposure to pups led Rosenblatt to conclude that the animals had a 'basic maternal responsiveness' which was not dependant on hormones (Rosenblatt, 1967). However, he concluded that the hormonal effect of pregnancy possibly contributed to the rapid onset of maternal behaviour at parturition.

1.4 Infanticide

In rats, mice and gerbils, the development of parental behaviour replaces infanticide which would be the usual response to pups. The reasons behind infanticide are as yet not fully understood, and cannot be generalised. Suggested reasons include the use of the pup as a food source, the removal of other individuals which would later be competing for resources, a quicker opportunity for a male to reproduce, reduction of the litter size to a 'manageable' size, manipulation of the sex-ratio, and the prevention of expending energy in pup care for infants not related to the adults (Elwood and Ostermeyer 1984b; Labov et al, 1985), or to ensure there was no waste of energy expenditure by adults in care-taking behaviour if litters were small and therefore not to be profitable (Wickler and Seibt, 1983). Work with mice has shown that the testing procedure employed to investigate infanticide can have an effect on the incidence of infanticide (McCarthy and vom Saal, 1986). Prolonged isolation of males (45 days) significantly inhibited infanticide when males were later tested in their home cages, although not when tested with a lactating female and her litter. With rats, the incidence of cannibalism has been shown to increase when pups are artificially stressed by methods such as neurosurgery or heat stress, or if the mother was traumatized for example by cage flooding (De Santis and Schmaltz, 1984). In hamsters, pup-cannibalism is interpreted as "an organized part of normal maternal behaviour", allowing a female to adjust her litter size depending on her capabilities in current environmental conditions (Day and Galef, 1977).

A study of naive male Mongolian gerbils (Elwood and Ostermeyer, 1984c) has suggested infanticide is enhanced by food deprivation, suggesting that males treat the pup as a food item. Increasing isolation of male gerbils prior to exposure to pups is seen to enhance infanticide, thought to be similar to the effect of dominance of one animal. It is possible that isolation allows a subordinate animal to recover from subordination and its effects on its behaviour. Since subordination inhibits infanticide, an animal recovered from the influence of this would be likely to show increased infanticide. A review of cannibalism (including infanticide) in several species is provided by Jones (1982).

1.5 Previous use of methods employed in current work

1.5 i Use of enclosed pups

The method used throughout this work, that is the presentation of enclosed pups to adults had been used before by other with varied results. The reason for the use of this method on previous occasions had not been to provide protection for pups but rather to investigate the role of selected cues from hidden pups. Roth (1967) found a longer latency to the development of maternal behaviour when adult rats were not exposed directly to pups but had them "next-door" in a wire basket hanging on the side of their cage. The only cues not present in this situation as compared with direct contact with pups were direct tactile cues. Jakubowski and

Terkel (1985) found no differences between control Albino rats exposed to an empty wire basket for 3 days and test animals exposed to pups in wire baskets for 3 days in the rate of the induction of parental behaviour at subsequent pup exposures. Koller (1952) found no increase in nest-building in female mice when pups were under a wire cover and therefore no tactile contact could be made, but did see the increase when pups were accessible (cited in Noirot, 1972b). Noirot (1966b) also failed to increase nest-building in adults by exposure to an inaccessible litter. Later Noirot, (1969a) carried out experiments with mouse pups enclosed in various metal boxes so as to produce only olfactory and auditory cues or auditory cues alone. Maternal behaviour (including nest-building) was subsequently produced. Noirot (1972b) concluded that the different cues selectively primed different maternal responses. Overall then, it seemed cues from protected pups would provide adequate stimulation to produce parental behaviour in naive adults.

Another factor related to pup cues stimulating parental behaviour is the influence of litters living in the same room as the experimental animals, which was found to have a significant effect with mice (Noirot, 1972b). The presence of pups nearby in the room seems likely to have at least some 'priming' effect on the surrounding adults. In the preliminary experiments in this work, the majority of gerbils showed an aggressive response to pups on their first direct contact encounter with a pup, so it seems the effect of neighbouring pups in the animal house was negligible.

1.5 ii Continuous exposure method

Continuous exposure to pups is a method which has been used by several authors, and as would be expected involves leaving pups in the adult's cage continuously, often with pups being replaced with 'fresh' ones at intervals (Wiesner and Sheard, 1933; Rosenblatt, 1967). Instead of continuous exposure, the method used throughout the present study involved 10 minute exposures to pups over days.

1.5 iii Stimuli for priming

Specific cues identified as emanating from pups in earlier work are discussed in more detail in later chapters. As can be seen from Figures 1.1 - 1.3, the appearance of pups changes rapidly with age. Consequently, visual cues will change with age. An obvious difference is the amount of body hair, clearly illustrated in Figures 1.1 - 1.3. The development of homiothermy and the rate of ultrasonic calling are thought to be inter-related (De Ghett, 1974), and change with the age of the pup. These changes are known to be related to parental behaviour (Broom et al, 1977). In order to avoid these effects and therefore to keep pup stimuli as constant as possible throughout all experiments, pups of a similar age and as young as possible were used throughout experiments.

Figure 1.1 Litter of one day old pups with their father.

Figure 1.2 Litter of seven day old pups with their father.

Figure 1.3 Litter of fourteen day old pups with their mother.

1.6 The Mongolian gerbil

The Mongolian gerbil, a cricetid rodent, was first introduced into the United Kingdom in 1964. Since then it has been extensively studied (Marston and Chang, 1965; Marston, 1976), and used increasingly in behaviour studies. As already noted, gerbils are known to live in extended family groups, that is a male and a female pair for breeding and stay together over several breeding seasons along with young from two or more litters (Elwood, 1983). The animals naturally live in underground burrows with a nest area and storage chambers. They were found naturally to be most active at dawn and dusk (see Marston, 1976). When kept in cages, obviously no burrow system can be developed. However a nest is built from the bedding material, sometimes totally enclosing the animals. When in captivity, the animals tend to be inactive duing the day, but will carry out their usual behaviour patterns if wakened.

Paired gerbils reproduce readily, with a gestation period of approximately 25 days. There is a post-partum oestrus, which if accompanied by successful fertilization, results in delayed implantation of the blastocysts as long as the first litter is suckling (Norris and Adams, 1971).

Studies of family groups in semi-natural conditions (Agren, 1978), suggested conclusions from laboratory studies were correct,

in that the animals did live as monogamous pairs with young of more than one litter. Territories were seen to be established, with chambered burrows being constructed. Only one female in a family group was seen to be breeding at one time, due to failure of sexual maturation in young females while living with their mother. The sexes appear to show division of labour, with males involved in territory defense, and females in hoarding. As in laboratory experiments, both sexes were involved in the care of the young. Seasonal and diurnal activities were also seen to be as predicted by laboratory experiments.

1.7 Aims of the study

The aims of the present study were to see whether or not full-scale parental behaviour could be produced in naive male and female Mongolian gerbils by exposure to protected pups. Also to investigate the infant characteristics underlying the development of this induced behaviour if it occurred. The characteristics to be investigated would include odour, visual and auditory cues. Behaviour frequencies shown would be compared between the sexes. Another aim was to study the maintenance of any induced parental behaviour over different lengths of time, comparable with the lactation period and beyond in order to compare induced parental responsiveness with parental behaviour seen in the laboratory situation with regard to their permanence.

CHAPTER TWO GENERAL METHODS

2.1 Sources of Animals

Animals used throughout experiments were Mongolian gerbils, Meriones unguiculatus, a cricetid rodent (Marston, 1976). They were bred from colony stocks at Dundee College of Technology (see section 2.1 iii Maintenance). These were also supplemented with animals from B.S. and S. Ltd, Edinburgh. Breeding pairs were established by pairing one male and one female, approximately three months old. Pairs were watched intermittently for approximately twenty four hours after introduction in case of fighting. If fighting occurred, the animals were separated and paired with a second, unfamiliar partner. Litters were weaned approximately twenty one days after birth. Offspring were then raised in littermate, single sex groups until paired for breeding or used as experimental animals. The majority of litters were born between the months of March and July, with an average litter size of 5.06 (see figures 2.1 and 2.2).

2.1 i Experimental animals

Animals were kept in male - female pairs during experiments. Stock animals were paired as described in section 2.1. All experimental pairs were nulliparous. They had had no experience of raising a litter, their only experience of pups having been with their own littermates. Pairing was carried out when adults were

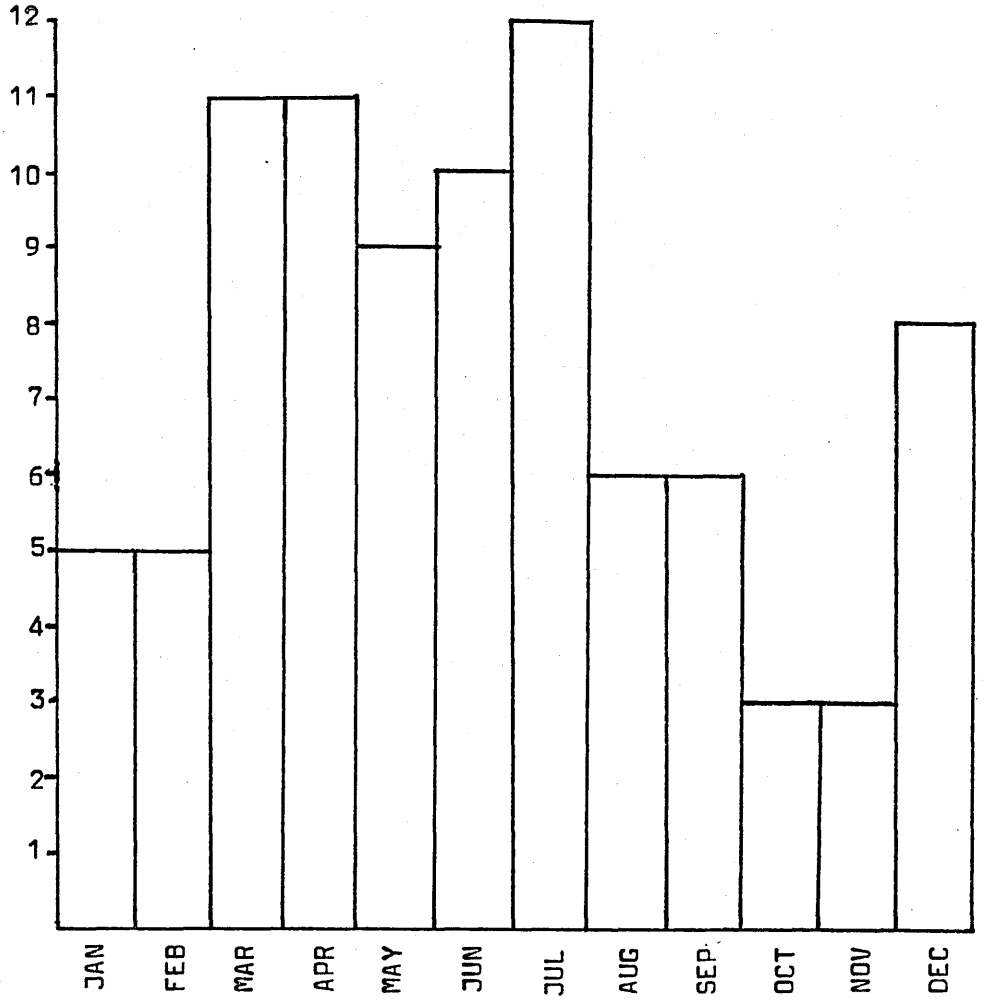

Figure 2.1

Numbers of Litters born each month between December 1983 - December 1985. (Average number of breeding pairs = 9)

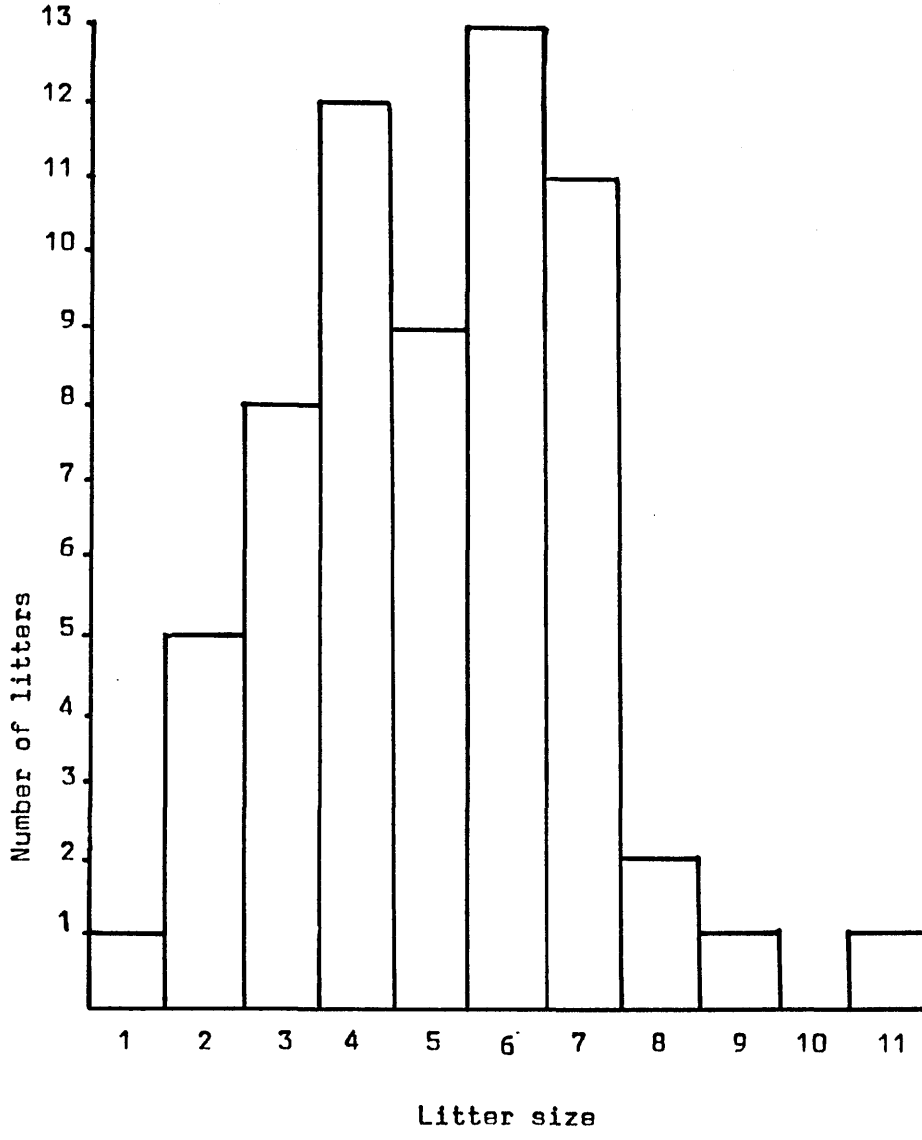

Figure 2.2 Sizes of litters born between December 1983 and December 1985.

between three and six months of age, with experiments starting within one or two weeks of pairing. Any pairs in which the female was subsequently found to be pregnant were discounted from the study due to known effects of the female's pregnancy on both members of the pair's parental behaviour (Elwood, 1977).

2.1 ii Stimulus Animals

Pups used as stimuli for behavioural tests were between 2-10 days of age, with 75% of these being 2-5 days of age. All pups were used, with the probability of using a male being 0.5.

2.1 iii Maintenance

Three cage sizes were available for use throughout experiments. Breeding pairs were kept in either 450 x 280 x 130mm or 380 x 260 x 200mm cages. Experimental pairs were kept in 380 x 260 x 200mm cages, although in one experiment, 350 x 150 x 130mm cages were used. Animals were kept under a twelve hour light-dark cycle, with lights on at 0800 hrs. All observations were carried out within the light period, between 0900 hrs and 1700 hrs. The environmental temperature was kept at approximately 21^{o}C. Food and water were available continuously. The animals were fed Labsure Diet CRMX, expanded rat and mouse diet, occasionally supplemented with sunflower seeds. Bedding consisted of woodshavings, with paper towel provided for the animals to shred for nest material.

2.1 iv Surgical Technique - Bilateral vasectomy

Anaesthesia was induced using an intraperitoneal injection of sodium pentobarbital (BDH Poole, England), 50mg per ml, in 0.8 -1.0ml of physiological saline (Marston, 1976) Animals were fully anaesthetized within 5 - 6 minutes, after which the testes were shaved, and bilateral incisions made, lateral to the ventral mid-line. Each testis in turn was brought to the exterior with the associated epididymis and the vas deferens. Approximately 5mm of the vas deferens was cauterised.

In closing the incisions, care was taken to repair both the peritoneal layer and the overlying fascia and skin. Braided silk, 5.0 gauge thread (Davis and Geck, London) was used with an atraumatic circle tapered needle.

During recovery, each gerbil was kept wrapped in paper towelling and placed on a heated steel table (Scientific and Research Instruments Ltd, Kent), set at 40^{o}C. Recovery from anaesthesia occurred within 2 - 4 hours. The recovery rate following this operation was 90%.

2.2 Materials

2.2 i Observation Chamber

An observation chamber measuring 550 x 381 x 262mm constructed from plywood, was placed over cages for observations (Figure 2.3). The top of the box contained a one-way plastic filter, measuring 416 x 272mm. The purpose of the filter was to prevent the animals being distracted by movements from above the chamber during testing. The filter was made effective by switching on the two white 30W strip lights inside the chamber. Twelve 10mm diameter holes were drilled in the chamber. These prevented an increase in temperature in the chamber from the heat of the lights. The temperature on the cage floor during observations was between 33 - 34.5^{o}C.

2.2ii Stimulus Pup's Protective Cage

A tubular cage (Figure 2.4) was made of 2 x 2mm gauge mesh, measuring 70mm long, with a diameter of 35mm and was attached to a flat mesh base, measuring 120 x 110mm. The tube itself was closed off and attached to the base with insulated wire.

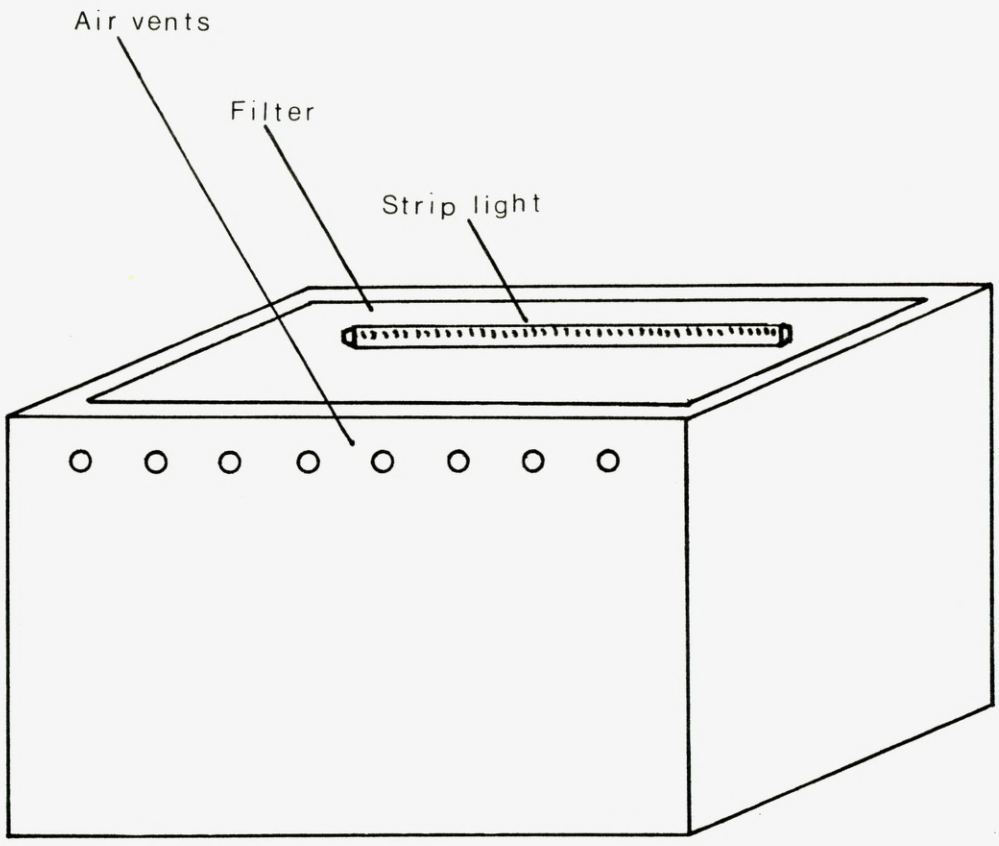

Figure 2.3 Observation Chamber.

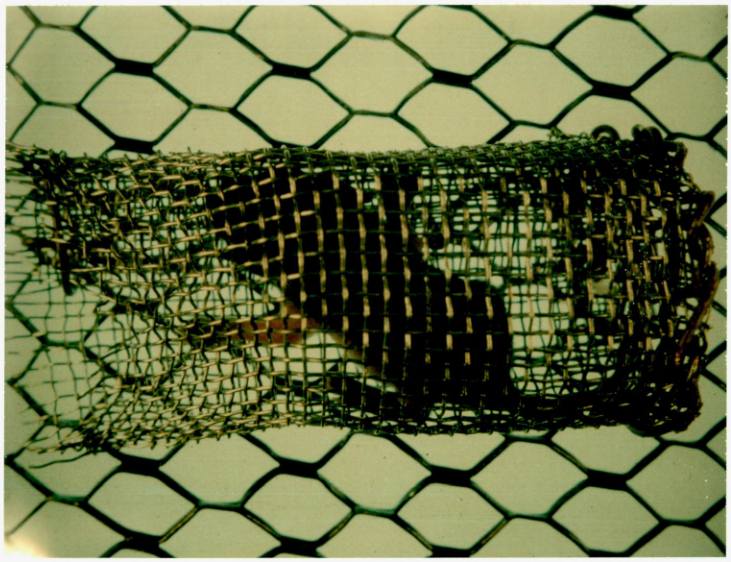

Figure 2.4 Stimulus pup's protective cage

2.3 General Behavioural Testing Procedure

All observations were carried out on individual animals within their home cages. Adult experimental animals were singly exposed to one of a variety of stimulus objects (a protected pup, PP, an unprotected pup, UP, (test) or an empty stimulus pup's protective cage, ('Control'), depending on the experiment in progress. (See Methods for each experimental chapter). The behaviour patterns shown by the adult during exposures were recorded on a checksheet, (Fig 2.5). Each adult was tested once per day.

Just prior to the beginning of an observation, the member of a pair not to be tested was removed from the home cage and placed in a holding cage for the duration of the observation of its partner. The cage top, food hopper and water bottle were removed from each cage prior to observations. The cage top was replaced by perspex and wire mesh. The perspex was used to cover the flat top of the cage, with the wire mesh being moulded to fit the sloping end of the cage (Fig 2.6). Bedding was left in the cage during observations. Two strips of paper were placed in the cage at the start of an observation as an additional source of nest material. New paper was put into each cage, and for each member of a pair if necessary (that is if either piece was chewed by the previous subject). In all cases, the stimulus object (that is protected pup / unprotected pup / stimulus pup's cage) was placed within the adult's cage at the opposite end from their nest. The observation chamber was placed over the cage, marking the beginning of the

Figure 2.5 Checksheet for recording behaviour pattern frequencies.

EXP. NO. / CAGE NO.	PUP NO. / AGE			SHEET NO. / TEST/CONTROL						DATE / TIME	
BEHAVIOUR \ TIME	1	2	3	4	5	6	7	8	9	10	COMMENTS
INACTIVE											
GROOM SELF											
SCRATCH CAGE											
FEED											
SNIFF OWN CAGE											
SNIFF MESH											
SNIFF PUP'S CAGE											
GNAW MESH											
GNAW PUP'S CAGE											
TEAR PAPER											
MARK CAGE											
MARK OTHER											
GNAW OWN CAGE											
BURROW											
BURROW UNDER PUP CAGE											

Figure 2.6 Covering of cage top during experiments.

exposure. More specific details of the method of 'test' and 'control' exposures are given in later chapters.

2.3 i Removal of Pups from the Parental Nest

The parents of a litter were enticed from the nest with food pellets or sunflower seeds, placed at the opposite end of the cage. A pup was quickly removed from the nest. The parents were again distracted for replacing pups, with the handled pup being placed under some of its littermates. This procedure prevented the parents being unduly disturbed, which might have led to the litter being neglected or cannibalized. If sufficient pups were available, no pup was used more than once per day. Re-use was limited to twice per day, with a minimum of 30 minutes between.

2.4 Recording of Results

2.4 i Checksheets

During the adults' exposures to stimulus objects, the behaviour patterns shown by the adults were recorded on checksheets (Fig. 2.5). These consisted of a grid, with the behaviour patterns recorded in the rows, and the sampling intervals recorded over the columns. The behaviour recorded for each sample interval was that which was shown predominantly over the 15s period. The occurrence of behaviours for short periods were recorded, but not included in the analysis. Additional details noted on the checksheet included date, time, experimental adult number, pup age and number, and the type of exposure being carried out. (Martin and Bateson, 1986).

2.4 ii Ultrasonics checksheets

Checksheets were also used to record ultrasonic vocalizations heard when played back at audible frequencies (see section 7.2) (Fig. 2.7). The number of calls heard in each 15 second period was recorded alongside the corresponding behaviour pattern.

2.5 Analysis of Results

In all experiments, results were summarized with one value, the median, being recorded for each individual for each group of exposures. These were the raw data used in statistical analyses. The median values of the above medians were calculated, and recorded in tables of results.

For statistical analysis, four tests were used (Siegel, 1956). These were the Kolmogorov-Smirnov two sample test, the Mann-Whitney U test, Wilcoxon's matched-pairs signed-ranks test, the Spearman rank correlation coefficient and the Fisher exact probability test.

Figure 2.7 Checksheet for recording ultrasonic vocalizations alongside corresponding behaviour patterns.

ULTRASONIC VOCALIZATIONS

EXP. PAIR NO. PUP NO. SHEET NO. DATE

CAGE NO. AGE PROT./UNPROT. TIME

TIME		ULTRASONICS	BEHAVIOUR	COMMENTS
MIN.	SEC.			
1	15			
	30			
	45			
	60			
2	15			
	30			
	45			
	60			
3	15			
	30			
	45			
	60			

CHAPTER THREE DESCRIPTIONS OF BEHAVIOUR PATTERNS / VOCALIZATIONS RECORDED

3.1 Behaviour Patterns

All the patterns described below refer to the adult animals. Reference is given to workers using the same or similar behaviour patterns. Patterns marked with an asterisk have been described as a parental pattern in rodents, or are closely related to the pup as a stimulus object.

INACTIVE The animal sat or stood motionless, and was not involved in any of the behaviour patterns described below. "Inactive" included the animal curled up and asleep. (Elwood, 1975).

GROOM SELF The animal cleaned its head / body / tail by rapidly rubbing itself with its forepaws. Often the animal nosed its ventral gland while grooming. "Groom self" also included the animal scratching its body, usually with one of its rear paws.

SCRATCH CAGE The animal rapidly scratched its cage wall with its forepaws, standing predominantly upright. (Elwood, 1975).

SNIFF OWN CAGE The animal sniffed around its cage with its nose visibly twitching. The animal was mobile or stationary.

VENTRAL GLAND MARKING The animal lowered its abdomen onto the cage floor to mark the floor with the sebum from its mid ventral scent gland. (Thiessen et al, 1969).

GNAW OWN CAGE The animal gnawed the mesh covering on the end of its cage (similar to "gnaw bars", Elwood, 1975).

BURROW The animal dug with its forepaws, moving the bedding from one area of the cage to another. The animal may simply have pushed bedding backwards and forwards.

BURROW/NEST-BUILD * The animal burrowed with its forepaws, at the same time curling and rotating its body, as if to hollow out a nest area. (Noirot, 1964). (Compare with 'burrow').

BURROW UNDER The animal dug bedding out from beneath the base of the stimulus pup's protective cage. The animal may have gone under the protective cage and continued burrowing.

SNIFF MESH * The animal sniffed the mesh base of the stimulus pup's protective cage.

SNIFF PUP'S CAGE * The animal sniffed the stimulus pup's protective cage.

GNAW MESH * The animal gnawed the mesh base of the stimulus pup's protective cage.

GNAW PUP'S CAGE * The animal gnawed the stimulus pup's protective cage.

TEAR PAPER * The animal held and shredded either or both of the strips of paper in its cage.

SIT OVER * The animal sat over the stimulus pup's protective cage.

SIT/LIE OVER PUP * The animal sat or lay over the top of the unprotected pup. (Makin and Porter, 1984). Other behaviour patterns may have been carried out simultaneously.

CHEW BEDDING * The animal picked up and chewed the bedding already present in the cage as distinct from the paper strips introduced at the beginning of the observation.

GATHER * The animal moved round its cage quickly, gathering up bedding in its mouth.

SNIFF/LICK PUP * The animal sniffed and/or licked the pup (Richards, 1966).

RETRIEVE * The animal rolled, pulled or carried the pup, usually to the nest area, then sat over the pup.

ARCH BACK * The animal sat over the pup with its back arched. (Similar to 'crouching', Krehbiel and Leroy, 1979; Makin and Porter, 1984).

BURY PUP * The animal collected bedding and placed it over the pup, or completely covered the pup with bedding when burrowing.

'INVESTIGATIVE PATTERNS' * Collective term including sniffing the pup's protective cage, the pup and the adult's own cage.

NEAR PUP The animal was $\leqslant$ 2.5cm from the pup.

3.2 Ultrasonic Vocalizations

These are calls from the animals, with a frequency between 16 - 80 kHz (Sales and Pye, 1974).

It was not distinguished whether calls were from the adults or the pups. Examples of visible representations in the form of sonagrams are shown in Chapter 7.

CHAPTER FOUR PRODUCTION OF PARENTAL RESPONSIVENESS IN NAIVE MALE AND FEMALE ADULT MONGOLIAN GERBILS.

4.1 Introduction

One of the initial aims of this work was to measure the responses of male and female adult gerbils to young pups, even though the adults had no experience of physically interacting with offspring. The first experiment described in this chapter was designed to see if indeed male and female animals showed any interest in young pups, even though they could not physically contact them, and whether the visual and olfactory experience gained from pups resulted in affiliative or even parental behaviour towards unprotected pups in later tests. Earlier work with Mongolian gerbils has shown that female pup inexperienced gerbils killed pups unless they were within six days of parturition. sixty percent of males killed pups unless their mate was pregnant. As described in chapter one, parental behaviour has been shown towards pups by inexperienced adults of several other rodent species (Beniest-Noirot, 1958; Wiesner and Sheard, 1933).

4.2 Method

Behavioural tests were carried out as described in section 2.3. For protected pup (PP) exposures, the stimulus objects were protected pups, while for control (C) exposures empty pup's protective cages were used. Eight males and eight females received 5 exposures to

pups in protective cages and 4 exposures to the cage alone. The sequence of exposures was random. At the end of the 9 exposures, all animals were exposed to an unprotected pup on one occassion. Each exposure lasted for 10 minutes unless in the UP exposure the experimental animal attacked the pup and then the test was immediately stopped before the pup was damaged. On the few occasions when pups were damaged, they were immediately killed by the animal technician.

Results were recorded as described in section 2.4, and are summarized in Tables 4.1 and 4.2. The results were analyzed using the Wilcoxon matched-pairs signed-ranks test and the Kolmogorov-Smirnov two sample test.

4.2 i Analysis of results.

For each individual animal, a median value of the frequency of 15s intervals spent in behaviour patterns was calculated for each behaviour pattern for each type of exposure, (PP or C). Statistical analysis was then carried out on these data. In order to compare protected pup exposures with control exposures, Wilcoxon's matched-pairs signed-ranks test was utilised. To compare males with females in either PP or C exposures, the Kolmogorov-Smirnov two sample test was used (Siegel, 1956).

Median values with interquartile values of the above medians were calculated. This gives one value for each behaviour for both protected pup exposures and control exposures for males and females. These medians of medians are the figures quoted in the majority of tables.

4.3 Results

4.3 i Comparison of PP with C exposures

4.3 i a Frequencies of behaviour patterns

Both males' and females' investigative patterns vary in frequency between protected pup (PP) and control exposures (C) (Tables 4.1 and 4.2). Males spent more time sniffing their own cage in C exposures than in PP exposures ($T = 4$, $p < 0.05$). Males spent more time sniffing the pup's cage in PP exposures than in C exposures ($T = 0$, $p < 0.01$). Similarly, females spent more time sniffing the pup's cage in PP exposures than in C exposures ($T = 0$, $p < 0.01$).

For both males ($T = 0$, $p < 0.05$) and females ($T = 1.5$, $p < 0.02$), more time was spent near the pup's cage in PP exposures than in C exposures. No differences were seen between PP and C exposures for males or females in the frequency of the occurrence of any of the other behaviours recorded.

4.3 i b Sex differences (PP and C exposures)

No significant differences were seen between males and females in PP exposures, or between males and females in C exposures for any of the patterns recorded, or in the amounts of time spent near the pup's cage.

Table 4.1 Comparison of frequencies of behaviour patterns shown by males (N = 8), in PP and C exposures.

Pattern	PP exposures *		C exposures *		p △
Inactive	14	(11.25-19.5)	18.75	(13.5-21.75)	
Groom self	4	(2.63-5)	4.25	(1.63-6.25)	
Scratch cage	0	(0-1)	0.25	(0-10.25)	
Sniff own cage	2.25	(2-3)	4	(3.25-4.75)	<0.05
Sniff mesh	0	(0-0.75)	0	(0-0.375)	
Sniff pup's cage	5	(3.25-7)	2	(0.5-2)	<0.01
Gnaw mesh	0	(0-0)	0	(0-0)	
Gnaw pup's cage	1.5	(0-3.38)	1.25	(0-5.25)	
Tear paper	2	(0-3.75)	2.25	(0-4.87)	
Mark cage	0	(0-0.75)	0	(0-0)	
Mark other	0	(0-0)	0	(0-0)	
Gnaw own cage	0	(0-0)	0	(0-0)	
Burrow	0	(0-0)	0	(0-0)	
Burrow under	0	(0-0)	0	(0-0)	
Gather nest material	0	(0-0)	0	(0-0)	
Chew bedding	0	(0-0)	0	(0-0)	
Sit over cage	0	(0-0)	0	(0-0)	
Total time ⩽ 2.5cm	7.75	(5.5-9.75)	3.75	(2.13-7.75)	<0.05

* median of medians with interquartile values in parentheses.

△ Wilcoxon's Matched Pairs Signed Ranks Test.

Table 4.2 Comparison of frequencies of behaviour patterns shown by females (N = 8), in PP and C exposures.

Pattern	PP exposures *	C exposures *	p △
Inactive	14.75 (10.25-18.13)	14.5 (12.25-17)	
Groom self	2 (0.13-5.38)	2.25 (1.25-3.38)	
Scratch cage	0.25 (0-1.25)	0 (0-5.63)	
Sniff own cage	2 (1-2.75)	3.25 (1.13-6.38)	
Sniff mesh	0 (0-0.875)	0.25 (0-1)	
Sniff pup's cage	4 (3.25-5.38)	1.25 (1-2)	< 0.01
Gnaw mesh	0 (0-0)	0 (0-0)	
Gnaw pup's cage	0 (0-2)	0 (0-0.75)	
Tear paper	7.5 (3.25-11.88)	8 (1.5-12.62)	
Mark cage	0 (0-0)	0 (0-0)	
Mark other	0 (0-0)	0 (0-0)	
Gnaw own cage	0 (0-0)	0 (0-0)	
Burrow	0 (0-0)	0 (0-0)	
Burrow under	0 (0-0)	0 (0-0)	
Gather nest material	0 (0-0)	0 (0-0)	
Chew bedding	0 (0-0)	0 (0-0)	
Sit over cage	0 (0-0)	0 (0-0)	
Total time ⩽ 2.5cm	6.5 (5.25-7.75)	2.75 (1.38-6.38)	< 0.02

* median of medians with interquartile values in parentheses.

△Wilcoxon's Matched Pairs Signed Ranks Test,

4.3 ii UP exposures

Times spent in various behaviour patterns during the unprotected pup (UP) exposures are shown in Table 4.3. Various behaviour patterns were shown which can be considered to be parental patterns. These include: grooming the pup (sniff/lick pup), which was shown by all the animals which had the one 10 minute UP exposure;

nest-building, which was shown by the three females which had the one 10 minute UP exposure;

sitting over the pup shown by two males and one female;

retrieving the pup, shown by one male and two females.

An observation not so far mentioned in results was that frequently in UP exposures, adults were seen to sniff the pup then run away from it.

Percentages of animals still responding aggressively to an unprotected pup are shown in Table 4.4, with 62.5% of both males and females responding aggressively.

SUMMARY

1. Experimental adults showed more interest in PP cages than C cages, in that investigative patterns increased during PP exposures, as did time spent near the pup's cage.
2. Some males and females showed parental patterns rather than aggressive responses to pups. This was possibly a result of PP exposures.
3. Sex-differences appeared to be present in some patterns in their frequency of occurrence, although never to a significant level.

Table 4.3. Frequencies of parental patterns shown by animals (♂N = 3; ♀N = 3) showing a non-aggressive response in UP exposure.

	Animal identification number					
Pattern	Males			Females		
	1	2	3	1	4	7
Sniff/lick pup	9	10	6	4	10	9
Tear paper	0	0	13	4	2	5
Chew bedding	0	0	0	4	0	0
Bury pup	0	0	1	0	0	0
Sit over pup	14	0	1	0	0	1
Gather	0	0	0	0	7	0
Retrieve	3	0	0	3	2	0
Burrow/N.B.	0	0	0	0	0	1
Sit over pup + groom self	0	0	0	0	0	1

Table 4.4. Percentages of animals still showing an aggressive response to an unprotected pup after a total of 5 PP and 4 Control exposures.

Males	Females
62.5	62.5

4.4 Discussion

4.4 i Comparison of PP with C exposures

A study of male and female pup-inexperienced adult gerbils' responses to test pups (Elwood, 1977) showed all non-pregnant females and 60% of males housed with pregnant females killed the pup, levels of aggression similar to those found here. Elwood noted that pups were killed in a manner similar to that used by the adults to take a piece of carrot, suggesting the pups were being treated as food. Studies here noted adults attacked pups. Since in this study increased interest was shown by the adults in the pup's protective cage when the pup was present in it compared with when it was empty, it is possible that any parental behaviour shown in the UP exposure resulted from the visual, odourous and perhaps vocal cues from the pup in the protective cage during the PP exposures, or similar cues from the pup in the UP exposure. The exposures to empty pup's cages seem unlikely to have affected behaviour in the subsequent UP exposure. It is possible that all females and 60% of males will never respond non-aggressively to pups on first exposure, and that in the current experiment no reduction in aggression was induced. However, it may be that the different methods used account for the different adult responses to pups.

Significantly, investigative patterns did show such a difference between PP and C tests. Elwood (1981) reported a sniffing response to test pups, which is similar to that seen during parturition, noting that the female then goes on to show several parental behaviours. This suggests that odour cues may be present on the pup which could mediate the development of parental behaviour. This idea is supported by several other authors, for example Fleming and Rosenblatt (1974b), who noted that approach/avoidance behaviour in rats tended not to occur until the female had sniffed foster pups. This idea is further investigated in Chapter 7.

A significant increase was also seen in the amounts of time spent near ($\leq$ 2.5cm) to the protective cage, in PP as compared to control exposures (that is an increase when the pup was present in the cage). This again implies that cues from the pup may be stimulating the change from an aggressive to a non-aggressive response in the experimental animal. Several cues may be available to the adult, including odour, visual and auditory cues. It seems reasonable to assume that all these cues would be readily detected by the adult at such close proximity to the pup. Consequently, the increase in time spent near the protective cage when it contained a pup may be associated with the loss of aggression towards an unprotected pup.

4.4 ii UP exposures

The behaviour patterns shown by adults during UP exposures included several which can be considered either to be parental behaviours or are patterns which could develop into parental behaviours. The parental patterns shown were groom pup, nest-build, retrieve and sit over pup. Sit over pup would more than likely develop into the arched back position. All six animals responding non-aggressively towards an unprotected pup showed at least one of the above parental patterns, with some individuals showing up to three. It is suggested that the amounts of time spent in these patterns would increase if UP exposures were increased. Since none of the animals showed a complete range of parental behaviours, it cannot be said that these six animals were fully parental, that is were primed, but it can be said that they were parentally responsive. It is likely that

priming would follow that is, a full range of parental patterns would be shown if unprotected pup exposures were increased.

The actual development of the parental behaviour appears not to be a unitary mechanism and probably occurs in two main stages: first the inhibition of aggression towards pups followed by a second stage where some parental patterns are induced, with others following with time, and increased pup exposure. Indeed frequently when observing the UP exposures, the animals were seen to sniff the unprotected pup then run away from it, implying that an aversion to the pup exists, and that this too must be overcome before the parental behaviour can fully develop. This in fact shows that each of the two main stages hypothesised above consist of a series of mechanisms which would lead finally to full parental responsiveness.

Although no sex differences were apparent in behaviours shown in any type of exposure, it is likely that some do exist, since male and female parents differ in their parental behaviour in laboratory studies (Elwood, 1975, 1977). For example, since only one UP exposure was carried out, it was not possible to detect whether males and females would differ with regard to how quickly they would respond non-aggressively over additional UP exposures. It is suggested that UP exposures allow the fear and aversion towards pups to diminish more quickly than PP exposures, so that increased UP exposures would show more animals developing first a non-aggressive, then a parental response to unprotected pups. Since the quality of a father and mother's behaviour is very similar, and the quantity shown depends on the presence of the other member of the pair, (Elwood, 1979a), (for

example nest-building is increased in one animal when the other member of the pair is not on the nest), perhaps sex-differences in frequencies of patterns will not be seen. Alternatively, sex-differences in amounts of behaviour are perhaps likely to be distorted here as compared with the laboratory situation since only one member of a pair is tested at any one time. This could cause slightly different results in comparison with those obtained in studies of pairs in the laboratory situation.

Future work arising from these results includes an investigation of the various cues emitted by the pups (Chapters 6 and 7). A more detailed study of the work reported here with increased UP exposures will investigate any differences between males and females.

CHAPTER FIVE DEVELOPMENT OF PARENTAL RESPONSIVENESS IN NAIVE MALE AND FEMALE ADULT MONGOLIAN GERBILS.

5.1 Introduction

Parental behaviour patterns were seen in some naive animals, possibly induced by short exposures to pups (Chapter 4). For example, after 5 exposures to protected pups, each of 10 minute duration, retrieving or arching over unprotected pups was induced in six animals (that is 37.5%). However, over half of the experimental animals were still aggressive to pups. This experimental chapter investigates whether a greater number of exposures to protected pups (PP) (a) inhibits aggression to pups in more animals and (b) induces a further display of parental behaviour.

As discussed in Chapter 4, it would be expected that sex-differences might be apparent in the rate of the development of parental patterns, therefore this too was investigated.

5.2 Method

Behavioural tests were carried out as described in section 2.3. For the duration of this test series, animals were housed in the 350 x 150 x 130mm high cages, which were smaller than those used in the experiments described in Chapter 4. The stimulus objects used were protected pups, and unprotected pups.

The test series was carried out in two blocks. The method varied slightly between the two blocks but where the exposure methods were similar, the data from all animals were taken together and analysed.

In block 1, nine pairs of animals were used. All animals were given 18PP exposures, then they were given 3UP exposures. Animals that had spent more than 25% of the time of PP exposures near ($\leqslant$ 2.5cm) the protected pup cage were given one UP exposure after 5, 10 or 15 PP exposures.

In block 2, five pairs of animals were used. The procedure was the same as that in Block I except that all animals received UP exposures after 5, 10, 15and 18 PP exposures, and when an animal showed a non-aggressive response to an unprotected pup, PP exposures were stopped, and a further two UP exposures were carried out.

In all cases if an aggressive response was shown to the unprotected pup during any of the UP exposures, the UP exposure was stopped.

In summary, animals received a maximum of 18 PP exposures (range 5 - 18), ultimately followed by three UP exposures.

5.2 i Analysis of results.

Results were recorded as described in section 2.4. For analysis of results, data were summarized as described in section 4.2.

Statistical tests used were the Mann-Whitney U test, Wilcoxon's matched-pairs signed-ranks test the Spearman rank correlation coefficient, and the Fisher exact probability test (Siegel, 1956).

In order to detect any changes in the animals' responses during the series of PP exposures, comparisons were made of medians of times spent in behaviour patterns over the first five PP exposures with medians of times spent in behaviour patterns over all PP exposures.

Frequencies of behaviour patterns recorded in intermediate UP exposures have not been compared, however their end results are described in the results since they do show some significant findings.

To detect whether or not increased PP and UP exposures did in fact increase either the percentage of animals responding non-aggressively and / or the range of parental patterns shown, results were compared between the two series of experiments described in Chapters Four and Five. Also, since animals were housed differently in work described in this chapter than in all other work described, these comparisons would detect any results which might be due to cage size rather than increased pup exposures, since apart from earlier 'control' exposures to empty pup's cages (considered not to affect resultant behaviour in the UP exposure), conditions were the same.

Spearman rank correlation coefficient was applied to data to see whether or not the frequencies of burrowing and aggressive responses were correlated.

5.3 Results

5.3 i PP Exposures

5.3 i a First 5 PP exposures

Frequencies of patterns, comparing males with females

Frequencies of behaviour patterns shown by males and females over the first five PP exposures (Table 5.1) showed sex differences in that females spent more time scratching the cage than males did ($U = 46$, $p < 0.02$), and males spent more time near the protected pup than females did ($U = 43.5$, $p < 0.02$).

5.3 i b All (18) PP exposures

Frequencies of patterns, comparing males with females

Over all 18 PP exposures (Table 5.2), no behaviour patterns differed significantly in frequency between males and females.

Correlation of burrowing / number of aggressive responses

There was no correlation between the frequency of burrowing/scratch cage and the number of aggressive responses shown in the final 3 UP exposures.

5.3 i c Comparisons of first five with all (18) PP exposures

Comparisons of times spent in behaviour patterns over the first five PP exposures with times spent in behaviour patterns over all PP exposures showed up several differences.

Table 5.1 Comparison of frequencies of behaviour patterns shown by males and females in first five PP exposures.

Pattern	Males*		Females*		p△
Inactive	0.5	(0-2.25)	1.0	(0-2.0)	
Groom self	0	(0-1.0)	1.0	(0-2.0)	
Scratch cage	0	(0-1.0)	2.0	(0-2.5)	<0.02
Sniff own cage	8.5	(5.25-9.25)	9.5	(6.0-10.25)	
Sniff mesh	0	(0-0)	0	(0-0)	
Sniff pup's cage	5.0	(3.75-6.0)	4.0	(3.0-5.25)	
Gnaw mesh	0	(0-0)	0	(0-0)	
Gnaw pup's cage	7.0	(5.0-14.25)	3.0	(0-13.5)	
Tear paper	0	(0-0)	0	(0-1.25)	
Gnaw own cage	0	(0-2.0)	0	(0-3.25)	
Burrow	0	(0-3.25)	0	(0-6.25)	
Burrow under	0	(0-2.5)	0	(0-0)	
Total $\leq$ 2.5cm	22.5	(18.0-26.25)	17.0	(13.0-22.0)	<0.02

* median of medians with interquartile ranges in parentheses.

△ Mann Whitney U test, using median values , comparing males with females.

Table 5.2 Comparison of frequencies of behaviour patterns shown by males and females over all PP exposures.

Pattern	Males*		Females*		p△
Inactive	0.5	(0-0)	0	(0-1.0)	
Groom self	0.75	(0-1.63)	1.25	(0-2.75)	
Scratch cage	0	(0-1.0)	1.25	(0-3.13)	
Sniff own cage	6.25	(5.0-9.0)	8.5	(6.88-9.5)	
Sniff mesh	0	(0-0)	0	(0-0)	
Sniff pup's cage	3.0	(2.0-4.0)	0	(0-0)	
Gnaw mesh	0	(0-0)	0	(0-0)	
Gnaw pup's cage	0.5	(0-4.75)	1.0	(0-2.13)	
Tear paper	0.75	(0-3.75)	0.25	(0-3.13)	
Gnaw own cage	0	(0-1.0)	0	(0-0)	
Burrow	3.5	(1.0-6.5)	4.0	(1.25-7.0)	
Burrow under	0.5	(0-2.5)	0	(0-6.25)	
Gather	0	(0-0)	0	(0-0)	
Chew bedding	0	(0-0)	0	(0-0)	
Sit over cage	0	(0-0)	0	(0-0)	
Total $\leq$ 2.5cm	17.25	(13.88-21.0)	16.5	(11.88-19.13)	

* median of medians with interquartile ranges in parentheses.

△ Mann-Whitney U Test, using median values, comparing males and females.

Frequencies of patterns

Males

Males (Table 5.3) spent significantly more time per exposure sniffing the pup's cage ($T = 4$, $p < 0.01$), gnawing the pup's cage ($T = 0$, $p < 0.01$) and more time near the pup's cage ($T = 4$, $p < 0.01$) in the first 5 PP exposures than over all exposures. They spent less time tearing paper ($T = 0$, $p < 0.01$) and burrowing ($T = 2$, $p < 0.01$) in the earlier PP exposures than over all PP exposures.

Females

Females (Table 5.4) spent more time inactive ($T = 0$, $p < 0.05$), gnawing the pup's cage ($T = 5.5$, $p < 0.02$) and gnawing own cage ($T = 0$, $p < 0.05$) in the earlier PP exposures than over all PP exposures.

5.3 ii Latency to a non-aggressive response.

Comparison of males with females

The latency to the first non-aggressive response shown by adults varied between males and females (Table 5.5) Males showed a non-aggressive response sooner than females. Overall, twice as many males as females showed non-aggressive responses, although this was not quite significant (♂10/14;♀4/10, $p = 0.056$)

Four pairs of animals (Block I) did not receive their first UP exposure until after 18PP exposures. Again twice as many males as females responded non-aggressively. The exact point at which some of these animals showed a non-aggressive response cannot be identified.

Table 5.3 Comparison of frequencies of behaviour patterns shown by males between first five and all PP exposures

Pattern	First 5 PP exps *		All PP exposures*		p Δ
Inactive	0.5	(0-2.25)	0.5	(0-2.13)	
Groom self	0	(0-1.0)	0.75	(0-1.63)	
Scratch cage	0	(0-1.0)	0	(0-1.0)	
Sniff own cage	8.5	(5.25-9.25)	6.25	(5.0-9.0)	
Sniff mesh	0	(0-0)	0	(0-0)	
Sniff pup's cage	5.0	(3.75-6.0)	3.0	(2.0-4.0)	< 0.01
Gnaw mesh	0	(0-0)	0	(0-0)	
Gnaw pup's cage	7.0	(5.0-14.25)	0.5	(0-4.75)	< 0.01
Tear paper	0	(0-0)	0.75	(0-3.75)	< 0.01
Gnaw own cage	0	(0-2.0)	0	(0-1.0)	
Burrow	0	(0-3.25)	3.5	(1.0-6.5)	< 0.01
Burrow under	0	(0-2.5)	0.5	(0-2.5)	
Total $\leqslant$ 2.5cm	22.5	(18.0-26.25)	17.25	(13.88-21.0)	< 0.01

* median of medians with interquartile ranges in parentheses.

Δ Wilcoxon matched-pairs signed ranks test, using medians.

Table 5.4 Comparison of frequencies of behaviour patterns shown by females between first five and all PP exposures

Pattern	First five PP exps*		All PP exposures*		p Δ
Inactive	1.0	(0-2.0)	0	(0-1.0)	< 0.05
Groom self	1.0	(0-2.0)	1.25	(0-2.75)	
Scratch cage	2.0	(0-2.5)	1.25	(0-3.13)	
Sniff own cage	9.5	(6.0-10.25)	8.5	(6.88-9.5)	
Sniff mesh	0	(0-0)	0	(0-0)	
Sniff pup's cage	4.0	(3.0-5.25)	0	(0-0)	
Gnaw mesh	0	(0-0)	0	(0-0)	
Gnaw pup's cage	3.0	(0-13.5)	1.0	(0-2.13)	< 0.02
Tear paper	0	(0-1.25)	0.25	(0-3.13)	
Gnaw own cage	0	(0-3.25)	0	(0-0)	< 0.05
Burrow	0	(0-6.25)	4.0	(1.25-7.0)	
Burrow under	0	(0-0)	0	(0-6.25)	
Total $\leq$ 2.5cm	17.0	(13.0-22.0)	16.5	(11.88-19.13)	

* median of medians with interquartile ranges in parentheses.

Δ Wilcoxon matched-pairs signed ranks test, using medians.

Table 5.5 Latency to first non-aggressive response shown.

UP Exposures		Numbers of animals non-aggressive	
Intermediate	Final	♂♂	♀♀
1 (after 5PP)		0/10	0/10
2 (after 10PP)		3/10	0/10
3 (after 15PP)		0/7	0/10
	1 (after 18PP)*	5/11	2/14
	2 (after 18PP+1UP)	2/6	2/12
		$p = 0.056^{\Delta}$	

* Four males and four females were not given a UP exposure until this stage, therefore their non-aggressive response may have developed at an earlier stage.

Δ Fisher exact probability test (♂ vs ♀)

Fractions : numerator = number of animals non-aggressive for the first time, denominator = number of animals not already having shown a non-aggressive response.

5.3 iii Intermediate UP exposures.

5.3 iii a Frequencies of patterns

Block I

In the first intermediate UP exposures (Table 5.5) after 5 PP exposures, five pairs of animals were given 1 UP exposure, and all responded aggressively. After 10 PP exposures the same five animals were again given 1 UP exposure. All females were aggressive, but one male responded non-aggressively, showing investigative behaviours. After 15 PP exposures, these same animals were given 1 UP exposure. The same male was non-aggressive, again showing investigative patterns.

Block II

Non-aggressive responses were shown by two males after 10PP exposures, with both showing investigative behaviours towards the pups. These animals then received a further two UP exposures. The 3 UP exposures together were considered as 'final 3 UP exposures'. (See section 5.2).

5.3 iv Final UP exposures.

5.3 iv a Frequencies of patterns

Comparison of males with females

In the final UP exposures, twice as many males as females showed

a non-aggressive response to the unprotected pup, significant only on UP 1 ($p = 0.046$) (Table 5.6). One behaviour pattern, burrowing, varied in frequency between males and females in the UP exposures (Table 5.7). Females spent more time burrowing than males ($U = 0.5$, $p < 0.002$). Some of the behaviour patterns earlier described as parental or pup-induced behaviours were shown by both males and females during the UP exposures. These included nest-building, sitting over the pup and burying the pup.

5.3 v Comparison of preliminary and current experiments

5.3 v a Latency to a non-aggressive response

In comparisons between the preliminary work (described in Chapter Four) and the present work, more animals, both males and females, showed a non-aggressive response to an unprotected pup in the present work than in the preliminary work, although never to a significant level (♂ $p = 0.27$; ♀ $p = 0.44$, Tables 5.8 and 5.9). In the current work, greater numbers of PP exposures were required by both males and females to produce the same level of non-aggressive responses to an unprotected pup as compared with preliminary work (Table 5.10). Between twice and almost four times as many PP exposures were required to produce a non-aggressive response in this experiment. Females at this stage had still not reached the level of parental responsiveness reached in preliminary experiments.

Table 5.6 Numbers of animals showing a non-aggressive response in the final three UP exposures

UP exposure	Numbers of animals non-aggressive		p*
	Males	Females	
UP 1	8/14	2/14	0.046
UP 2	10/14	4/14	0.056
UP 3	9/14	5/14	0.12

* Fisher exact probability test.

Fractions - numerator = number of animals showing a non-aggressive response.

denominator = number of animals tested.

Table 5.7 Comparison of frequencies of behaviour patterns shown by males and females in the final UP exposures

Pattern	Males*		Females*		p △
Inactive	1.0	(0-2.63)	0	(0-0.25)	
Groom self	2.0	(0.75-4.0)	3.0	(0.25-3.75)	
Scratch cage	0.5	(0-4.0)	1.5	(0.5-4.0)	
Sniff own cage	9.0	(6.5-11.13)	11.0	(8.0-12.25)	
Sniff/lick pup	9.25	(8.0-14.0)	8.5	(4.0-12.75)	
Tear paper	1.0	(0-5.63)	0	(0-1.5)	
Gnaw own cage	0	(0-2.25)	0	(0-1.0)	
Burrow	0.75	(0-2.25)	12	(6.0-18.0)	<0.002
Chew bedding	0	(0-0)	0	(0-0)	
Bury pup in nest	0.75	(0-1.5)	1.0	(0-3.5)	
Sit over in nest	0.5	(0-3.75)	0	(0-2.5)	
Burrow/nest-build	0	(0-0.25)	0	(0-0)	
Sit over + NB	0	(0-0.38)	0	(0-0)	
Total $\leq$ 2.5cm	15.0	(13.25-25.0)	12.5	(9.5-17.5)	

* median of medians with interquartile ranges in parentheses.

△ Mann-Whitney U test.

Table 5.8 Numbers of animals showing aggressive and non-aggressive responses in UP exposures (preliminary experiment).

Sex	Non-aggressive (inc. parental)	Aggressive
♂	3/8	5/8
♀	3/8	5/8

Table 5.9 Numbers of animals showing aggressive and non-aggressive responses in UP exposures (current experiment)

Sex	Non-aggressive (inc. parental)	Aggressive
♂	10/14	4/14
♀	9/14	5/14

Comparison between levels of aggression between preliminary and current experiments

p = 0.27*

p = 0.44

* Fisher exact probability test.

Fractions - numerator = number of animals showing the particular response

denominator = number of animals tested

Table 5.10 Percentages of animals showing a non-aggresssive response after 5, 10, 15 and 18 PP exposures, and after 18PP + 1 UP exposures in preliminary and current experiments.

	Males		Females	
	Preliminary	Current	Preliminary	Current
5PP	37.5% = 3/8[Δ]	0% - 0/10	37.5% = 3/8	0% = 0/10
10PP	*	30% = 3/10	*	0% = 0/10
15PP	*	0% = 0/10	*	0% = 0/10
18PP	*	45% = 5/11	*	14% = 2/14
18PP + 1UP	*	33% = 2/6	*	16.7% = 2/12

* Animals in preliminary experiment were given a maximum of 5 PP exposures.

Δ Numerator of fraction represents the number of animals showing a non-aggressive response. Denominator represents the number of animals given a UP exposure.

5.3 v b Comparison of first five PP exposures

Frequencies of patterns

Data from the first five PP exposures were compared between results of preliminary and current work (Tables 5.11 and 5.12). In the current work males spent more time sniffing their own cage ($U = 6$, $p < 0.002$), gnawing the pup's cage ($U = 10.5$, $p < 0.002$), and in total time spent near ($\leq$ 2.5cm)the pup's cage ($U = 0$, $p < 0.002$) and less time self-grooming ($U = 4$, $p < 0.002$). Correspondingly, females spent more time sniffing their own cage ($U = 1.5$, $p < 0.002$) and in total time near the pup ($U = 6.5$, $p < 0.002$), and less time inactive ($U = o$, $p < 0.002$) and tearing paper ($U = 11$, $p < 0.002$).

5.3 v c Comparison of final UP exposures

Parental behaviour patterns shown in the final UP exposures did not vary in frequency between preliminary and current experiments (Tables 5.13 and 5.14).

SUMMARY
1. Sex differences were shown in the frequencies of behaviour patterns in the first 5 PP exposures, with females showing more 'scratch cage' than males, and males spending more time 'near pup' than females.
2. Males showed more investigative behaviour patterns in the earlier PP exposures, and developed possible parental patterns (tearing paper) in later PP exposures, accompanied by increased burrowing.
3. Females showed more investigative behaviour patterns in the earlier PP exposures than in later ones.
4. Only males responded non-aggressively towards pups during intermediate UP exposures. Parental patterns were shown, including 'sit over pup' and nest-building.
5. Males showed a shorter latency to a non-aggressive response to an unprotected pup than females did.

Table 5.11 Comparison of frequencies of behaviour patterns shown by males in first five PP exposures between preliminary and current experiments.

Pattern	Preliminary*		Current*		p△
Inactive	14.0	(11.25-19.5)	0.5	(0-2.25)	<0.002
Groom self	4.0	(2.63-5.0)	0	(0-1.0)	<0.002
Scratch cage	0	(0-1.0)	0	(0-1.0)	
Sniff own cage	2.25	(2.0-3.0)	8.5	(5.25-9.25)	<0.002
Sniff mesh	0	(0-0.75)	0	(0-0)	
Sniff pup's cage	5.0	(3.25-7.0)	5.0	(3.75-6.0)	
Gnaw mesh	0	(0-0)	0	(0-0)	
Gnaw pup's cage	1.5	(0-3.38)	7.0	(5.0-14.25)	<0.002
Tear paper	2.0	(0-3.75)	0	(0-0)	
Gnaw own cage	0	(0-0)	0	(0-2.0)	
Burrow	0	(0-0)	0	(0-3.25)	
Burrow under	0	(0-0)	0	(0-2.5)	
Total $\leqslant$ 2.5cm	7.75	(5.5-9.75)	22.5	(18.0-26.25)	<0.002

* median of medians with interquartile ranges in parentheses.

△ Mann-Whitney U test.

Table 5.12 Comparison of frequencies of behaviour patterns shown by females in first five PP exposures between preliminary and current experiments.

Pattern	Preliminary*		Current*		p△
Inactive	14.75	(10.25-18.13)	1.0	(0-2.0)	<0.002
Groom self	2.0	(0.13-5.38)	1.0	(0-2.0)	
Scratch cage	0.25	(0-1.25)	2.0	(0-2.5)	
Sniff own cage	2.0	(1.0-2.75)	9.5	(6.0-10.25)	<0.002
Sniff mesh	0	(0-0.875)	0	(0-0)	
Sniff pup's cage	4.0	(3.25-5.38)	4.0	(3.0-5.25)	
Gnaw mesh	0	(0-0)	0	(0-0)	
Gnaw pup's cage	0	(0-2.0)	3.0	(0-13.5)	
Tear paper	7.5	(3.25-11.88)	0	(0-1.25)	<0.002
Gnaw own cage	0	(0-0)	0	(0-3.25)	
Burrow	0	(0-0)	0	(0-6.25)	
Burrow under	0	(0-0)	0	(0-0)	
Sit over	0	(0-0)	0	(0-0)	
Total $\leq$ 2.5cm	6.5	(5.25-7.75)	17.0	(13.0-22.0)	<0.002

* median of medians with interquartile ranges in parentheses.

△ Mann-Whitney U test.

Table 5.13 Comparison of frequencies of behaviour patterns shown by males in UP exposures between preliminary and current experiments.

Pattern	Preliminary*		Current*		p Δ
Sniff/lick pup	9.0	(6.0-10.0)	9.25	(8.0-14.0)	
Chew bedding	0	(0-0)	0	(0-0)	
Bury pup in nest	0	(0-1.0)	0.75	(0-1.5)	
Sit over in nest	1.0	(0-14.0)	0.5	(0-3.75)	
Retrieve	0	(0-3.0)	0	(0-0)	
Sit over + NB	0	(0-0)	0	(0-0.38)	
Burrow/nest-build	0	(0-0)	0	(0-0.25)	
Total $\leq$ 2.5cm	10.0	(8.0-27.0)	15.0	(13.25-25.0)	

* median of medians with interquartile ranges in parentheses.

Δ Mann-Whitney U test.

Table 5.14 Comparison of frequencies of behaviour patterns shown by females in UP exposures between preliminary and current experiments.

Pattern	Preliminary*		Current*		p Δ
Sniff/lick pup	9.0	(4.0-10.0)	8.5	(4.0-12.75)	
Chew bedding	0	(0-4.0)	0	(0-0)	
Bury pup in nest	0	(0-0)	1.0	(0-3.5)	
Sit over in nest	0	(0-1.0)	1.0	(0-3.5)	
Gather	0	(0-7.0)	0	(0-0)	
Retrieve	2.0	(0-3.0)	0	(0-0)	
Burrow/nest-build	0	(0-1.0)	0	(0-0)	
Total $\leq$ 2.5cm	10.0	(7.0-12.0)	12.5	(9.5-17.5)	

* median of medians with interquartile ranges in parentheses.

Δ Mann-Whitney U test.

6. Parental behaviour patterns were shown in the final UP exposure by both sexes, although males' were in greater number.
7. Greater percentages of animals responded non-aggressively to pups in this experiment as compared with the preliminary one, however there was a longer latency to a non-aggressive response in this experiment.
8. Both males and females spent more time near the protected pup in the first five PP exposures of this experiment than in the preliminary experiment. Investigative behaviours were also more frequently shown by both sexes in the current experiment than in the preliminary one.

5.4 Discussion

5.4 i PP exposures.

5.4 i a First five

Frequencies of patterns comparing males with females

Sex differences were found when frequencies of behaviours shown by males and females in the first 5 PP exposures were compared. Females were found to scratch the cage more than males. Elwood (1975) suggested scratching the cage was part of the burrowing behaviour pattern. The pattern may indeed be part of the animals' natural burrowing behaviour (Rich, 1968) or may be an attempt to escape from the cage, either due to disturbance because of the presence of the protected pup in the cage, or as a result of fear. The fact that females show this behaviour more than males do, and that there is no significant sex difference in the frequency of the pattern over all PP exposures supports the idea suggested earlier that the development of the parental response occurs in stages. This particular result supports the idea of a second stage in the development of parental responsiveness, that is a decrease in aversion to pups over exposures, following a loss of aggression. A

sex difference is apparent, with males reaching this second stage of the process sooner than females. No correlation was found between the frequency of burrowing/scratching cage and the number of aggressive responses in the final 3 UP exposures.

5.4 i b Comparison of first five with all PP exposures.

From the comparisons of frequencies of behaviour patterns occurring at the 5 PP exposure stage with those over all PP exposures it can be seen that over the duration of the PP exposures, differences occurred in the frequencies of some patterns which also suggest that different stages occur in the development of parental responsiveness, with different patterns being representative of the different stages. Males showed more investigative behaviours, that is sniffing and gnawing the pup's cage in the early PP exposures than they did over all PP exposures. This suggests investigation of the pup occurs to begin with, but as exposures are continued, the adult does not need to spend as much time investigating the pup. This could be due either to habituation to a novel stimulus, or could be due to the males recognizing the familiarity of the stimulus, and progressing onto the next stage of the development of parental responsiveness to pups. This stage would not involve as much investigative behaviour as the earlier stage. Males also spent more time near the pup's cage in the earlier PP exposures. This is likely to be for the same reasons as discussed above. The increase in time spent tearing paper in the later PP exposures (shown by an increase

overall compared with first 5 PP exposures) is probably an increase in parental behaviour, in the form of nest-building. This shows parental responsiveness is developing. The increase in time spent burrowing in the later PP exposures may be a parental pattern, possibly part of the more complex behaviour pattern of nest-building. The greater time spent inactive by females in the earlier PP exposures is probably indicative of fear. As the female overcomes her fear during subsequent PP exposures, she would become more active. Gnawing the pup's cage is an investigative behaviour, the greater occurrence of this pattern in the earlier PP exposures showing that the females are undergoing a change in their behaviour, from fear to investigative. This change is probably slower than the same change in the male, since little or no fear (as suggested by inactivity) is shown by the males during early PP exposures, and since males show other investigative behaviors as well as gnawing the pup's cage. Gnawing own cage is comparable with 'gnaw bars' as described by Elwood (1975). He suggests this may be an attempt to escape due to disturbance from the pups. A further new suggestion is that this may be a pattern produced as a result of fear, due to the presence of the pup in the adult's cage.

5.4 ii Latency to a non-aggressive response.

Males showed a shorter latency than females to a non-aggressive response in UP exposures. This gives an indication that males overcome their fear of and/or aggression towards pups sooner (that is after less pup contact) than females do. Laboratory studies on the loss and re-establishment of pup cannibalism when a female is

pregnant (Elwood 1977, 1980) have shown males lose their aggression earlier in the females pregnancy than the females do. It is thought males are responding to cues from the pregnant female, possibly progesterone metabolites in her urine. The change in the female is thought to be due to a 'specific physiological state'. A similar result of the shorter latency of males found here is unlikely to be due to the same mechanisms since their partners were not pregnant. Induced parental responsiveness by exposure to pups may require less stimulation for males than females.

For six animals of four pairs, the exact point of their non-aggressive response cannot be identified from the results, since they had no opportunity of contact with an unprotected pup until after 18 PP exposures. However, one female never responded non-aggressively, and one showed a non-aggressive response on the second of the series of 3 UP exposures, showing it is unlikely that the results from these four pairs are opposing the results of the other ten pairs.

5.4 iii UP Exposures

Sex-differences did occur in the numbers of animals responding non-aggressively to unprotected pups in UP exposures, with more males than females responding non-aggressively. This supports the idea suggested in Chapter Four that the development of parental behaviour is a non-unitary response, with the gradual development through stages occurring more quickly in males than in females. Here it seems males overcome their aggression to pups more quickly than females do,

that is, the first stage in the development of parental behaviour occurs more quickly in males than in females. The similar result found in laboratory studies is discussed above in section 5.4 ii. As already said, it is unlikely that the hormone changes associated with pregnancy occur in the development of parental responses in naive adults by exposure to pups, therefore it is possible some other factor exists which causes males to lose their aggression to pups sooner than females, both in the case of a pair consisting of a primiparous female and a naive male, and with a naive pair being exposed to pups.

The frequency of patterns shown during UP exposures varied in one pattern only between the sexes. Females spent more time than males engaged in burrowing. As discussed earlier, the burrowing behaviour described here may well be part of nest building, but is distinct (section 3.1) from the pattern described as burrow/nest build. Again it seems that this greater burrowing shown by the females may be due to the presence of the pup disturbing the female. The fact that males showed less of this burrowing seems to support the idea that males undergo the transition in the development of the parental response more quickly than females. This is also supported by males showing this increased burrowing over the series of PP exposures, implying the same disturbance effect is seen in males, but earlier in the exposure series. When considering the results of PP exposures in conjunction with the results of UP exposures, it appears the difference between males and females in their latency to lose their aggression may well be apparent during PP exposures.

5.4 iv Comparison of preliminary with current experiments

5.4 iv a Comparison of latency to non aggressive response

When results were compared between the preliminary experiment reported in Chapter four and the current experiment reported above, more animals showed parental behaviour patterns to unprotected pups in current experiments, but with a longer latency. Terkel and Rosenblatt (1971) reduced the latency to a non-agressive response to pups in naive female rats by reducing the cage floor area, concluding the smaller area made it difficult for the female to avoid the pup, so that fear was overcome. Similarly Vestal and Hellack (1977), working with male white footed mice, found male-male interactions in a small arena were likely to end in investigatory responses to each other, as opposed to avoidance seen in larger test areas. This was thought to be a direct effect of the test area, since animals could not avoid each other, and so investigated each other. From this, and since cage sizes used in the current experiments fall within the range (that is between their smallest and largest) used both by Terkel and Rosenblatt and Vestal and Hellack, a similar result would have been expected that is, increased investigative behaviours, leading to a shorter latency. However, the opposite occurred, since a longer latency to a non-aggressive response occurred with smaller cage size. Possibly the cage was so small that the adults were forced into close proximity of the pups, and fear prevented the investigative behaviour. However, at the end of the series of exposures, no differences are found in the frequency and range of

behaviour patterns shown between animals housed in large and small cages. The experiment did show however, that increased PP and UP exposures increased the percentages of animals responding non-aggressively.

5.4 iv b Comparison of first five PP exposures

In the first five PP exposures of the two experiments, both males and females showed a decrease in times spent inactive from the larger cage area to the smaller area, showing overall activity increased. Cage size is the more likely of the two differences between the methods used to have caused this, as the presentation of empty cages to adults seemed not to affect results (discussed in Chapter 4). Elwood (1975) has noted that mothers' activity increased with increasing litter age due to 'disturbance' from the pups. It is possible that the increase in activity here is due to the adults being forced closer to the pups due to the smaller cage size. This could result in 'disturbance' in the adults, giving an overall increase in activity. Both males and females showed an increase in time spent sniffing their own cage when in smaller cages. Conversely, Walters, Pearl and Rogers (1963) reported gerbils showed an increase in exploratory behaviour with increasing test area, although this was also thought to be related to the 'reward value' of the environment. It is possible that the presence of the pup increases investigation of the environment, and since in a smaller area the adults have difficulty avoiding the pup, the end result is an increase in sniffing the adult's cage. Both males and females showed a decrease in time spent tearing paper from preliminary to current experiments. This suggests parental patterns had not developed at this stage in

the current experiment, when the animals were housed in the smaller cages. This would appear to be the case, since a longer latency to a non-aggressive response to an unprotected pup was shown by the animals in the smaller cages. Males and females showed an increase in time spent near the pup when housed in smaller cages. This is almost certainly a direct result of cage size. Males showed a decrease in self grooming when housed in smaller cages. It is unlikely that this is related to the development of the parental response. Males also showed an increase in time spent gnawing the pup's cage when housed in smaller cages. The cause of this pattern is unclear but may be investigatory.

5.4 iv c Comparison of behaviour frequencies in UP exposures

No differences were seen in the frequencies of patterns shown in UP exposures when comparing the results of the two experiments. This implies the end result of exposure to pups is unaffected by cage size. Since the latency to a non-aggressive response to a pup is different however, there are possibly differences in the frequencies of patterns shown during the PP exposures. From these results it seem increased PP and UP exposures do not increase the range of parental behaviours shown in the UP exposures.

Overall, increased PP and UP exposures did increase the percentages of animals responding non-aggressively. The range of parental patterns shown was not increased.

CHAPTER SIX PUP STIMULI I - ODOUR.

6.1 Introduction

Having established that parental behaviour could be produced in naive adult Mongolian gerbils of both sexes by exposure to young pups, procedures were designed to investigate the role of any cues from the pups which could be involved in the development of the behaviour. Cues available from the pups could be visual, auditory and olfactory. Maternal behaviour has been produced in naive mice by exposure to hidden pups (Noirot, 1969a) so that it seems visual cues are not essential, if at all important in the priming process in mice.

The odour of infant rodents is thought to play an important role in the development of parental behaviour. It is known that the odour of a rat pup aids a mother in identifying her own young when they are among strangers (Beach and Jaynes, 1956).

In Mongolian gerbils it has also been shown that adults scent-mark their pups with the sebum from their mid-ventral scent gland, and that this serves to identify the members of their own litter (Wallace, Owen and Thiessen, 1973).

Other authors have demonstrated that the young of some rodent species are marked with urine, and that this serves to identify them, and enhances the male's tolerance of young (Kleiman, 1974). 'Nest odours', that is the odours in the nest

(to which the female would have contributed), have also been suggested as 'marking' pups, and thus helping to identify a female's own litter (Beach and Jaynes, 1956). Presumably the female's urine would contribute a large part to the make-up of the 'nest-odour'.

Since the gerbil pups used here in experiments are living in a nest made up of material which is extremely likely to absorb urine excreted by the parents, it seems the parents' urine could also be contributing to 'labelling' the pups.

It is also known that the odour of a pup can inhibit a non-lactating rat from approaching and maintaining close contact with the pup (Rosenblatt et al, 1979). It is also likely that the pup with its own odour would be aversive to a naive adult.

Work with rats has demonstrated that novel stimulation could evoke both fear and the 'exploratory drive', resulting in approach/avoidance behaviour towards the stimulus (Montgomery, 1955). While the stimuli used were not pups, presumably pups would be included among novel stimuli to naive adults, and could therefore produce a fear in the adults resulting in approach/avoidance behaviour towards pups. Removal of the olfactory bulb in adult mice eliminated maternal behaviour in both virgin and lactating mice, implying that the olfactory system is involved in the development of maternal behaviour (Gandelman et al 1971).

In contrast, naive adult female rats rendered anosmic become parental more quickly than intact females (Fleming and Rosenblatt, 1974a). This was taken as an indication that pup odours could delay the onset of maternal behaviour in virgins. This theory has also been confirmed for gerbils (Clark, Spencer and Galef, 1986), with females that tended to avoid contact with novel olfactory stimuli also showing more aggression towards pups and less retrieval of pups (retrieval is often taken as indicative of the development of maternal behaviour), suggesting the unaltered odour of the pup evokes fear in the naive female. It was also shown that in gerbils, as in rats, the rate of induction of maternal behaviour by concaveation is in part mediated by the response of naive adults to novel stimuli from pups. Work described here earlier (Chapters 4 and 5) has suggested that investigative behaviours are important in the development of parental responsiveness.

An experiment was therefore designed to investigate whether or not sebum and urine from the adults, when used to mark pups, were effective in overcoming the fear of a naive adult, and allowing parental behaviour to develop. If this was found to be the case, it was also planned to investigate if the two were as effective as each other in reducing the latency to a non-aggressive response to an unprotected pup.

6.2 Method

All males of pairs used in this work were vasectomized prior to the start of testing, The method of the vasectomy is described in section 2.1 iv.

The odours of the pups to which the adults were exposed were altered to be the same as that of either the experimental adult's scent gland sebum ('sebum experiments') or urine ('urine experiments'). To do this, pups were removed from the parental nest as described in section 2.3 i. Their backs were then washed with distilled water, and dried with cotton wool. For 'sebum experiments' the experimental adult was held up with its scent gland exposed, and the pup was then rubbed on the scent gland to transfer the odour of the adult to the pup. At the end of the exposures, the pups were again washed, and were then rubbed on either of their parents' scent glands before being returned to the parental nest. For 'urine experiments' the procedure was similar, except that pups had urine from the experimental adult smeared on their backs in place of scent gland sebum. Cotton-wool pledgets were used to smear the urine on the pups.

Urine was collected by isolating the experimental adults in empty cages. Pools of urine were collected with a Pasteur pipette, and stored in sealed vials at 4^{o}C until required.

Exposures were otherwise carried out as described in section 2.3. All adults received 3 PP exposures, then were given 1 UP

exposure (See Figure 6.1). All animals which attacked pups had the UP exposures stopped and were given one further PP exposure. This was repeated for all adults until they were non-aggressive in order to determine the exact point at which aggression stopped. Once they responded non-aggressively, each adult received a total of 10 UP exposures in the 'sebum' experiment, or a total of 3 UP exposures in the 'urine' experiment. If an animal reverted to an aggressive response after 2 or more UP exposures, that UP exposure was stopped. The animal received no further PP exposures, but was given further UP exposures on subsequent days, up to a total of 10. Control exposures were those in which the stimulus pups did not have their odour altered. Ten pairs were used in each odour experiment.

For analysis of results, frequencies of behaviour patterns were summarized by calculating a median value for each animal for each type of exposure. Comparisons were made of behaviour pattern frequencies between the sexes, between 'sebum' and 'urine' experiments and between test and control experiments. In control experiments pups bore their natural odour. The latency to a non-aggressive response was also compared between sexes, between 'sebum' and 'urine' tests, and between test and control experiments. For all analyses the statistical test used was the Mann-Whitney U test.

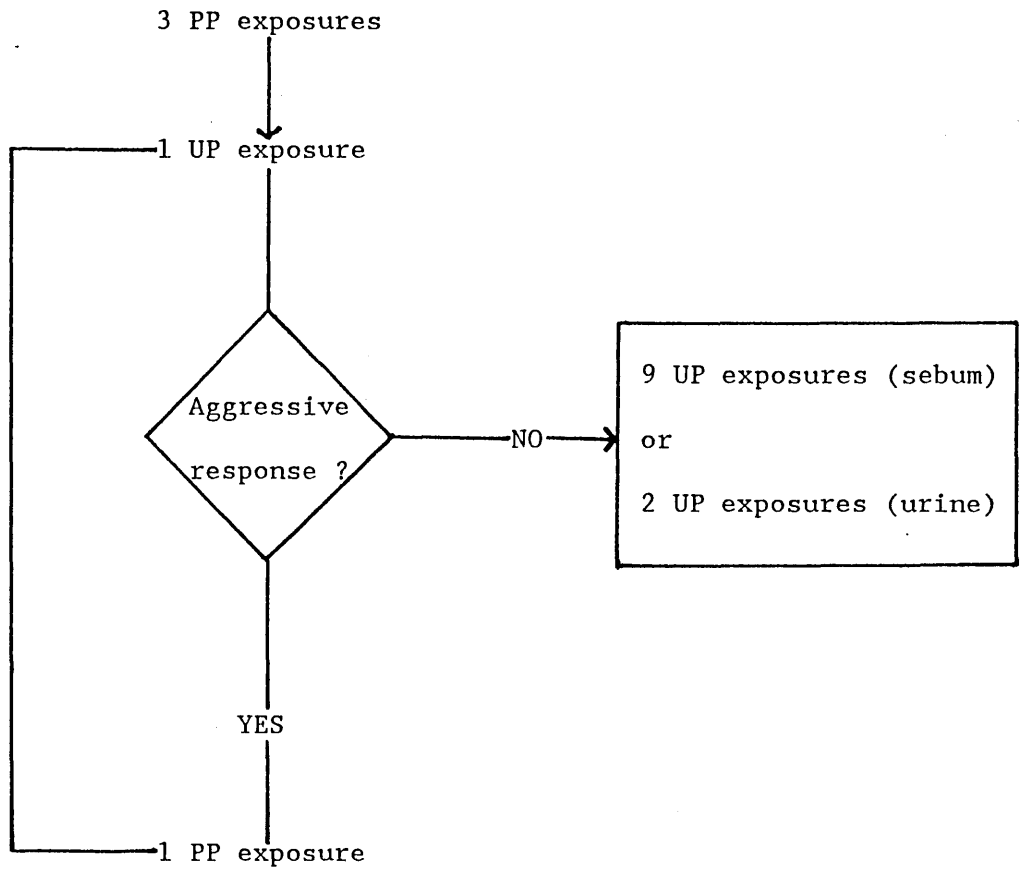

Figure 6.1 Sequence of exposures in 'sebum' and 'urine' experiments.

6.3 Results

6.3 i Sebum results

6.3 i a UP Exposures

These were compared both for sex differences in behaviour pattern frequencies at different stages of the series of UP exposures, and for behavioural pattern frequency differences shown at different stages of the series. The latency to a non-aggressive response was also compared between the sexes.

Sex differences occurring over UP exposures

Males and females showed no differences in the frequency of behaviour patterns shown during the first 5 or over all UP exposures (Tables 6.1 and 6.2). However, males showed significantly more pup-sniffing in the first three UP exposures than over all UP exposures (Table 6.3) ($U = 19$, $p < 0.02$). Females showed no differences (Table 6.4).

Latency to a non-aggressive response

When the numbers of PP exposures required before a non-aggressive response was shown in UP exposures were compared between males and females, it was found that males required significantly less PP exposures than did females (Medians : ♂ = 3, ♀ = 4; $U = 20$, $p < 0.05$; Table 6.5).

Table 6.1 Comparison of frequencies of behaviour patterns shown by males and females over first 5 'sebum' UP exposures

Pattern	Males*		Females*		p Δ
Inactive	1.0	(0-2.25)	0	(0-1.88)	
Groom self	0.5	(0-1.0)	1.0	(0-2.25)	
Scratch cage	0	(0-1.0)	1.0	(0-2.75)	
Sniff own cage	14.5	(11.0-16.5)	10.0	(8.0-15.25)	
Sniff pup	8.0	(7.75-9.0)	7.5	(4.75-10.0)	
Tear paper	1.5	(0-4.75)	1.5	(0-4.25)	
Burrow	2.5	(0.75-5.5)	2.5	(0-6.25)	
Sit over	0.5	(0-1.25)	0	(0-0)	
Gather	0	(0-1.25)	0	(0-0)	
Total $\leq$ 2.5cm	14.5	(13.0-16.25)	14.0	(11.0-19.5)	

* Median of medians with interquartile values in parentheses.

Δ Mann-Whitney U test.

Table 6.2 Comparison of frequencies of behaviour patterns shown by males and females over all (10) sebum UP exposures

Pattern	Males*		Females*		p$^{\Delta}$
Inactive	1.0	(0-2.0)	0	(0-1.125)	
Groom self	0	(0-1.0)	0.5	(0.38-2.0)	
Scratch cage	0	(0-0.625)	1.0	(0-3.5)	
Sniff own cage	12.0	(9.62-15.25)	10.0	(8.5-11.75)	
Sniff pup	7.0	(5.38-8.25)	5.0	(4.0-6.0)	
Tear paper	0	(0-2.0)	1.75	(0-3.63)	
Gnaw own cage	0	(0-0)	0	(0-0)	
Burrow	0.75	(0-4.62)	3.25	(0-5.0)	
Sit over	0.5	(0-1.0)	0	(0-0.125)	
Sit over + NB	0	(0-0)	0	(0-0)	
Gather	0	(0-1.13)	0	(0-0)	
Total $\leq$ 2.5cm	14.25	(13.25-16.25)	12.5	(10.63-14.5)	

* Median of medians with interquartile values in parentheses.

$^{\Delta}$Mann-Whitney U test.

Table 6.3 Comparison of frequencies of behaviour patterns shown by males over all UP exposures with first three UP exposures in 'sebum' experiment.

Pattern	First 3 UPs*		All UPs*		p△
Inactive	1.0	(0-2.0)	1.0	(0-2.0)	
Groom self	0.5	(0-1.25)	0	(0-1.0)	
Scratch cage	0	(0-1.0)	0	(0-0.625)	
Sniff own cage	15.0	(10.75-17.25)	12	(9.62-15.25)	
Sniff pup	9.0	(7.75-11.0)	7	(5.38-8.25)	< 0.02
Tear paper	1.5	(0.75-6.25)	0	(0-2.0)	
Burrow	2.5	(1.0-6.0)	0.75	(0-4.62)	
Sit over	0	(0-1.0)	0.5	(0-1.0)	
Gather	0	(0-1.25)	0	(0-1.13)	
Total < 2.5cm	13.5	(12.75-16.25)	14.25	(13.25-16.25)	

* Median of medians with interquartile values in parentheses.

△ Mann-Whitney U test.

Table 6.4 Comparison of frequencies of behaviour patterns shown by females over all UP exposures with first three UP exposures in 'sebum' experiment.

Pattern	First 3 UPs*		All UPs*		p △
Inactive	0	(0-2.0)	0	(0-1.125)	
Groom self	1.0	(0-2.25)	0.5	(0.38-2.0)	
Scratch cage	1.0	(0-4.25)	1.0	(0-3.5)	
Sniff own cage	10.0	(8.0-15.25)	10.0	(8.5-11.75)	
Sniff pup	8.0	(4.0-10.25)	5.0	(4.0-6.0)	
Tear paper	1.5	(0-3.25)	1.75	(0-3.63)	
Burrow	2.5	(0-9.25)	3.25	(0-5.0)	
Sit over	0	(0-0.25)	0	(0-0.125)	
Gather	0	(0-0)	0	(0-0)	
Total $\leq$ 2.5cm	15.0	(12.75-20.25)	12.5	(10.6-14.5)	

* Median of medians with interquartile values in parentheses.

△ Mann-Whitney U test.

Table 6.5 Comparison of numbers of PP exposures required to produce a non-aggressive response to an unprotected pup in 'sebum' experiment.

Pair	Number of PPs required	
	♂	♀
1	3	5
2	3	4
3	3	3
4	3	3
5	3	4
6	3	5
7	3	5
8	3	5
9	3	3
10	3	4
p*	< 0.05	

* Mann-Whitney U test.

6.3 i b Test compared with control

Behaviour frequencies in UP exposures

In a comparison of frequencies of behaviour patterns after 3 UP exposures (since 'control' went no further) between test (sebum) and control (Table 6.6), males showed more burrowing ($U = 22.5$, $p < 0.05$) and spent more time sniffing their own cage ($U = 10.5$, $p < 0.02$) in test conditions. Females showed no differences between test and control (Table 6.7).

Latency to a non-aggressive response

It was found that significantly fewer PP exposures were required to produce a non-aggressive response to an unprotected pup when the pup bore the experimental adult's sebum than in the experiment when it did not. This was positive for both males and females (Medians : ♂T = 3, ♂C = 19; ♀T = 4, ♀C = 21; $U = 0$, $p < 0.002$; Table 6.8).

6.3 ii Urine results

6.3 ii a UP exposures (males compared with females)

These were compared for sex differences in behaviour pattern frequencies over all (3) UP exposures, and the latency to a non-aggressive response.

Table 6.6 Comparison of frequencies of behaviour patterns shown by males in first 3 (sebum) UP exposures and control UP exposures.

Pattern	First 3 UPs*		Control*		p Δ
Inactive	1.0	(0-2.0)	1.0	(0-2.63)	
Groom self	0.5	(0-1.25)	2.0	(0.75-4.0)	
Scratch cage	0	(0-1.0)	0.5	(0-4.0)	
Sniff own cage	15.0	(10.75-17.25)	9.0	(6.5-11.13)	< 0.02
Sniff pup	9.0	(7.75-11.0)	9.25	(8.0-14.0)	
Tear paper	1.5	(0.75-6.25)	1.0	(0-5.63)	
Burrow	2.5	(1.0-6.0)	0.75	(0-2.25)	< 0.05
Gather	0	(0-1.0)	0	(0-0.25)	
Sit over	0	(0-1.25)	0.5	(0.5-3.75)	
Total ⩽ 2.5cm	13.5	(12.75-16.25)	15.0	(13.25-25.0)	

* Median of medians with interquartile values in parentheses.

Δ Mann-Whitney U test.

Table 6.7 Comparison of frequencies of behaviour patterns shown by females in first 3 (sebum) UP exposures and control UP exposures.

Pattern	First 3 UPs*		Control*		p$^{\Delta}$
Inactive	0	(0-2.0)	0	(0-0.25)	
Groom self	1.0	(0-2.25)	3.0	(0.25-3.75)	
Scratch cage	1.0	(0-4.25)	1.5	(0.5-4.0)	
Sniff own cage	10.0	(8.0-15.25)	11.0	(8.0-12.25)	
Sniff pup	8.0	(4.0-10.25)	8.5	(4.0-12.75)	
Tear paper	1.5	(0-3.25)	0	(0-1.5)	
Burrow	2.5	(0-9.25)	12.0	(6.0-18.0)	
Gather	0	(0-0)	0	(0-0)	
Sit over	0	(0-0.25)	0	(0-2.5)	
Total $\leq$ 2.5cm	15.0	(12.75-20.25)	12.5	(9.5-17.5)	

* Median of medians with interquartile values in parentheses.

$^{\Delta}$ Mann-Whitney U test.

Table 6.8 Comparison of numbers of PP exposures needed to produce a non-aggressive response to an unprotected pup in sebum and control experiments.

Number of PP exposures			
♂		♀	
Sebum	Control	Sebum	Control
3	19	5	21+
3	21+	4	21+
3	10	3	19
3	21+	3	21+
3	10	4	21+
3	21+	5	19
3	10	3	21+
3	19	5	21+
3	18	3	20
3		4	21+
			21+
p* < 0.002		< 0.002	

* Mann-Whitney U test.

Sex differences occurring over all (3) UP exposures

Results showed (Table 6.9) that males and females differ in times spent in three behaviour patterns in UP exposures when the pups bore the urine of the experimental adult. Males spent more time inactive than did the females ($U = 7$; $p < 0.03$). Males also spent more time than the females self-grooming ($U = 5$; $p < 0.012$), and more time than the females sniffing their own cage ($U = 0.5$, $p < 0.001$).

Latency to a non-aggressive response

No significant sex-difference was seen between males and females in the number of PP exposures required to produce a non-aggressive response to an unprotected pup (Medians : ♂= 5.5, ♀= 5.5; Table 6.10).

6.3 ii b Test compared with control

Behaviour frequencies in UP exposures

Frequencies of behaviour patterns shown by males (Table 6.11) varied between test (urine) and control in time spent sniffing own cage, with a greater time spent in this pattern in test than in control UP exposures ($U = 10$, $p < 0.02$). Females showed no differences (Table 6.12).

Latency to a non-aggressive response

There was also a significant difference between the number

Table 6.9 Comparison of frequencies of behaviour patterns shown by males and females in 'urine' UP exposures.

Pattern	Males*		Females*		p△
Inactive	1.0	(1.0-2.75)	0.5	(0-2.0)	< 0.03
Groom self	0.75	(0-1.0)	0	(0-0.125)	< 0.01
Scratch cage	0	(0-1.75)	2.75	(0.75-6.75)	
Sniff own cage	13.5	(13.0-17.25)	8.75	(5.25-12.25)	< 0.001
Sniff pup	7.5	(6.25-9.0)	6.25	(5.0-8.63)	
Tear paper	0	(0-1.75)	0.25	(0-4.0)	
Burrow	1.5	(0-3.13)	3.5	(1.88-15.88)	
Sit over + NB	0	(0-2.25)	0	(0-3.0)	
Retrieve	0	(0-0)	0	(0-0.125)	
Gather	0	(0-1.5)	0	(0-0.63)	
Sit over	0	(0-0.375)	0	(0-1.38)	
Sit over + groom	1.25	(0-2.75)	0	(0-3.25)	
Total $\leq$ 2.5cm	17.0	(13.13-18.75)	14.25	(11.0-16.88)	

* Median of medians with interquartile values in parentheses.

△ Mann-Whitney U test.

Table 6.10 Comparison of numbers of PP exposures needed to produce a non-aggressive response to an unprotected pup in urine experiment.

Pair	Number of PPs required	
	♂	♀
1	3	4
2	3	3
3	6	5
4	3	4
5	4	10
6	6	8
7	7	10
8	5	10
9	10	5
10	10	5
p	NS	

Table 6.11 Comparison of frequencies of behaviour patterns shown by males in 'urine' UP exposures and controls.

Pattern	Urine*		Control*		p △
Inactive	1.0	(1.0-2.75)	1.0	(0-2.63)	
Groom self	0.75	(0-1.0)	2.0	(0.75-4.0)	
Scratch cage	0	(0-1.75)	0.5	(0-4.0)	
Sniff own cage	13.5	(13.0-17.25)	9.0	(6.5-11.13)	< 0.02
Sniff pup	7.5	(6.25-9.0)	9.25	(8.0-14.0)	
Tear paper	0	(0-1.75)	1.0	(0-5.63)	
Burrow	1.5	(0-3.13)	0.75	(0-2.25)	
Sit over	1.5	(0-5.63)	0	(0-0.25)	
Gather	1.0	(0-2.75)	0.5	(0-3.75)	
Total ≤ 2.5cm	17.0	(13.13-18.75)	15.0	(13.25-25.0)	

* Median of medians with interquartile values in parentheses.

△ Mann-Whitney U test.

Table 6.12 Comparison of frequencies of behaviour patterns shown by females in 'urine' UP exposures and controls.

Pattern	Urine*		Control*		p$^{\Delta}$
Inactive	0.5	(0.5-2.0)	0	(0-0.25)	
Groom self	0	(0-1.125)	3.0	(0.25-3.75)	
Scratch cage	2.75	(0.75-6.75)	1.5	(0.5-4.0)	
Sniff own cage	8.75	(5.25-12.25)	11.0	(8.0-12.25)	
Sniff pup	6.25	(5.0-8.63)	8.5	(4.0-12.75)	
Tear paper	0.25	(0-4.0)	0	(0-1.5)	
Burrow	3.5	(1.88-15.88)	12.0	(6.0-18.0)	
Sit over	0	(0-1.38)	0	(0-2.5)	
Gather	0	(0-0.63)	0	(0-0)	
Total $\leq$ 2.5cm	14.25	(11.0-16.88)	12.5	(9.5-17.5)	

* Median of medians with interquartile values in parentheses.

Δ Mann-Whitney U test.

of PP exposures required to produce a non-aggressive response to an unprotected pup when comparing the results from the urine exposures with the 'control' exposures, (Table 6.13) This was shown for both males and females (Medians : ♂T = 5.5, ♂C = 19; ♀T = 5.5, ♀C = 21; ♂U = 3; ♀U = 0, $p < 0.002$), with less PP exposures required when the pup bore the experimental adult's urine than when it had its own natural odour.

6.3 iii Comparison of 'Sebum' with 'Urine' experiments

Behaviour frequencies in UP exposures

When times spent in behaviours in UP exposures were compared between sebum marked pups (first three UP exposures) and urine marked pups no significant differences were found.

Latency to a non-aggressive response

Results of numbers of PP exposures required to produce a non-aggressive response to an unprotected pup were compared for males and for females, comparing results obtained when the pups bore the experimental adult's scent gland sebum, and those obtained when the pups bore the experimental adult's urine (Table 6.14). Results showed a significant difference for both males (Medians : sebum = 3, urine = 5.5; U = 15, $p < 0.02$) and females (Medians : sebum = 4, urine = 5.5; U = 23, $p < 0.05$), with fewer PP exposures required when scent gland sebum was used, than with urine.

Table 6.13 Comparison of numbers of PP exposures required to produce a non-aggressive response to an unprotected pup in urine and control experiments.

Number of PP exposures			
♂		♀	
Urine	Control	Urine	Control
3	19	4	21+
3	21+	3	21+
6	10	5	19
3	21+	4	21+
4	10	10+	21+
6	21+	8	21+
7	10	10+	20
5	19	10+	21+
10+	18	5	21+
10+		5	19
			21+
p*	< 0.002		< 0.002

* Mann-Whitney U test.

Table 6.14 Comparison of numbers of PP exposures needed to produce a non-aggressive response to an unprotected pup in sebum and urine experiments.

Number of PP exposures			
♂		♀	
Sebum	Urine	Sebum	Urine
3	3	5	4
3	3	4	3
3	6	3	5
3	3	3	4
3	4	4	10
3	6	6	8
3	7	3	10
3	5	5	10
3	10	3	5
3	10	4	5
p*	< 0.02		< 0.05

* Mann-Whitney U test.

SUMMARY

1. Males showed more pup-sniffing in the first three UP exposures than over all UP exposures.
2. In the sebum experiment, males required fewer PP exposures than females to produce a non-aggressive response in UP exposures.
3. Males showed more burrowing and sniffed their own cage more in the first three UP exposures in 'sebum' than in control experiments.
4.Both males and females required less PP exposures to produce a non-aggressive response in UP exposures in 'sebum' than in control experiments.
5. Males spent more time than females inactive, self-grooming and sniffing their own cage in 'urine' UP exposures.
6. Males sniffed their own cage more in 'urine' than in control UP exposures.
7. Both males and females required less PP exposures to produce a non-aggressive response in UP exposures in 'urine' than in control experiments.
8. Adults showed a shorter latency to a non-aggressive response to 'sebum' pups than to 'urine' pups.

6.4 Discussion

6.4 i Sebum experiment

It is interesting to note that the only behaviour pattern differing in frequency between all UP exposures and the first three UP exposures between the sexes is sniffing the pup (more of this behaviour pattern was shown in the first three UP exposures than over all UP exposures by the males). Investigative behaviours towards the pup were earlier noted as being significant in the development of a non-aggressive response to pups, but had not previously shown any sex-differences. Since it is the males which are showing this difference in frequency of sniffing of the pup, and since males also require significantly less PP exposures to allow a non-aggressive response to an unprotected pup to develop

than do females, this clearly indicates that sniffing of the pup is an important behaviour in the production of a non-aggressive response. This is in agreement with several authors who have shown that sniffing pups is important in the transition from non-maternal (including aggressive) to maternal behaviour. In virgin rats, sniffing pups seems to overcome an aversion to the pups' odour (Mayer and Rosenblatt, 1975). In mice, Noirot and Goyens (1971) noted that pregnant females showed a positive change in sniffing pups to which they were exposed as gestation progressed. A similar trend was noted in gerbils (Elwood, 1977), with pregnant females sniffing a pup then their own genital regions during exposure to pups. They then went on to display maternal behaviour towards the pup.

Since no sex-differences were seen in frequencies of behaviour patterns over the first 5 UP exposures, or when comparing the first 5 UP exposures with all UP exposures, but since the frequency of sniffing varied in males between the first three UPs and all UPs, it seems that males had developed more investigative behaviour before the females did, probably showing males are progressing more quickly through this stage of the development of parental responsiveness. The fact that females needed more PP exposures than males to produce a non-aggressive response to an unprotected pup also suggests aggression is lost more quickly in males than in females.

In sebum experiments, UP exposures were continued until animals had each had ten, in order to see if more parental

behaviour patterns would develop, or if their frequencies would increase with increased time in UP exposures. More patterns were in fact seen, and in greater fequency than before. However none of the differences seen were significant.

Males showed significantly more burrowing in test conditions than in control conditions. In earlier work (Chapter 5) this pattern was thought to be a result of disturbance due to the presence of the pup. As females do not show this increase in burrowing, it suggests that males are further on than the females in the stages of the transition from an aggressive to a parental response. Males also showed more sniffing of their own cage in test than in control experiments. As discussed earlier, this increase in investigative behaviour is indicative of the stage in the above transition after the overcoming of fear and aversion to pups. This stage had not been reached in the control experiments, therefore it can be concluded that the odour of the adult's sebum on the pup speeds up the progression of the stages in the development of parental responsiveness. Females did not show these differences, suggesting that at the 3 UP stage of test conditions, females still have not overcome their fear of the pup, and consequently their response is no different than in control conditions.

The fact that fewer PP exposures were required to produce a non-aggressive response in a UP exposure in test than in control exposures in both males and females also shows the odour of the adult's sebum on the pup speeds up the loss of aggression

preceding the developed parental responsiveness.

Since in this experiment the pup's natural odour had been altered to match the odour of the scent gland of the experimental adult, this suggests that in the natural situation the pups might be marked by their parents. Wallace et al (1973) showed that gerbil pups smeared with the sebum from a female's scent gland were more likely to be retrieved and cared for by that female than pups which had no scent, having been washed. Wallace et al also showed an increase in females' scent marking during pregnancy, and concluded that scent marking of pups would aid the mother in identifying her pups. Marking in non-pregnant females has been shown to be regulated by ovarian hormones (Owen and Thiessen 1973, 1974) as has the size of the scent gland (Blum et al, 1975).

The difference in times spent sniffing the pup between males and females in UP exposures could indicate females have a higher level of fear than the males, and this would therefore take longer to be overcome, therefore delaying the increase in investigatory patterns seen previously (Chapters 4 and 5). The difference could also be a consequence of the less-aggressive response of males to pups reported earlier both here (Chapter Five) and by Elwood (1980).

As mentioned in the introduction, novel odours of strange pups can inhibit the development of parental behaviour in naive females. This has been demonstrated both with rats (Fleming and Rosenblatt, 1974b) and gerbils (Clark et al, 1986). Montgomery

(1955) has shown that novel stimulation of rats evokes fear. A pup bearing an adult's own odour would presumably evoke less fear than a pup bearing a strange odour. It seems therefore that the reduced latency to a non-aggressive response towards an unprotected pup found in this work could be due to a loss of the fear brought about by the pup bearing the experimental adult's odour. Montgomery and Monkman (1955) showed fear as a result of novel stimulation did not invoke exploratory behaviour, which adds support to this since if fear is overcome, exploratory or investigative behaviour would proceed, leading to parental responsiveness. Fleming and Luebke (1981) have suggested that in rats the hormones which allow maternal behaviour to proceed at parturition could also decrease the females' fear responses. The natural development of maternal behaviour is due to changes in circulating hormone levels and pup-induced behaviour effectively by-passes hormone level changes. It therefore seems the overcoming of the fear response may also be hormonally mediated during pregnancy, but it may also be able to be overcome without the hormonal changes when adults are exposed to pups.

6.4 ii Urine experiment

The greatest difference seen between males and females was in the time spent sniffing own cage in UP exposures, with males showing this behaviour more than the females. As noted earlier on several occassions, this is an investigative behaviour. This adds support to earlier conclusions that investigation of the environmental odours, including the pups' odour, plays an

important part in the development of parental behaviour. Since males showed more of this behaviour than the females did, it is possible that the female may not be investigating the environment due to fear resulting from the presence of the pup at this stage. Females showing more overall activity than males may indicate disturbance due to the presence of the pups. The difference in time spent self-grooming may be due to the males distinguishing the odour of their urine from the odour of their sebum (which is spread over their bodies during self-grooming). As with the 'sebum' experiment, fewer PP exposures were required to produce a non-aggressive response to pups in UP exposures, again suggesting that an odour which is familiar to the adult speeds up the overcoming of aggression/fear of the pup.

6.4 iii Sebum/urine

No difference between frequency of behaviour patterns in UP exposures in urine and the first three sebum UP exposures were found. This suggests the familiar odour only helps overcome fear/aggression to pups, and does not affect the quality of behaviour produced.

In comparing the effectiveness of urine with that of sebum in reducing the latency to a non aggressive response to an unprotected pup, it was found that sebum produced a shorter latency to non aggressive behaviour than urine did with both males and females. As discussed earlier, it seems that if the pup bears an odour familiar to the adults, then the latency to a non

aggressive response will decrease. This is thought to be due to a novel odour, for example a pup's own odour, evoking fear in the adult, and the fear producing avoidance behaviour (Montgomery, 1955; Clark et al, 1986.)This fear is overcome when the pup bears a familiar odour. It is possible that the nest material around the pups does not have much urine absorbed in it, and therefore will not be passing the 'urine odour' onto pups. This might mean that sebum (which will be on pups) is 'more familiar'.

Another possible explanation for gerbil scent gland sebum being more effective in reducing the latency to a non aggressive response than urine, is that sebum is known to be used to identify a mother's litter (Wallace, Owen and Thiessen, 1973) and in other aspects of communication for example, in marking territory (Thiessen et al, 1970). Urine is not thought to be used by gerbils in communication, for example in territory marking, although it is used by other rodents (Bishop and Chevins, 1986). It is possible that sebum is more effective since the gerbils are familiar with their own scent from self grooming, whereas urine is only recognized as a familiar odour from the home cage. Sebum may reduce fear and promote parental behaviour. Urine may only reduce fear.

Although the quality of parental behaviour produced when stimulated by 'urine pups' and 'sebum pups' does not seem to differ, one extra point noted which is worth mentioning is that 'urine pup' stimulated adults were more likely to revert to aggression after one or two successful UP exposures, whereas on

the whole, once a 'sebum pup' stimulated adult had shown a non aggressive response this rarely reverted to aggression. This also supports the idea that sebum is more effective at producing parental behaviour in naive adults, and is therefore more likely to be one of the factors involved in the natural situation.

CHAPTER SEVEN PUP STIMULI II - ULTRASONIC VOCALIZATIONS.

7.1 Introduction

In addition to investigating the role of odour cues from pups in the development of parental behaviour, experiments were also carried out to investigate the role of auditory cues. Auditory cues are known to be produced by pups (Sewell, 1968), and to have an influence on producing maternal behaviour in several rodent species (Zippelius and Schleidt, 1956, Noirot, 1972a, Sewell, 1970a,b, Sales and Pye, 1974, Elwood, 1979b). Noirot (1972a) has suggested that two types of call exist in pups of several rodent species, one produced when pups are cold, resulting in increased nest-building by the adult, the other type being produced in response to rough handling, inhibiting the adults from doing this.

The cues produced are both auditory and ultrasonic calls, (in terms of the human hearing range) with the majority falling in the ultrasonic range. Ultrasonic sounds are those above the human hearing range. The optimum of human hearing is at approximately 2kHz, disappearing both below and above at 20Hz and 17 - 20kHz (Sales and Pye, 1974). Gerbil pups have been shown to emit ultrasonic calls between 16 and 80kHz . Hearing ranges of the adults have been shown to coincide with the call range. The calls are thus considered as being of probable communicatory significance (Brown, 1973b and Ralls, 1967).

It would therefore be interesting to record any ultrasonic vocalizations emitted during exposures to pups, and to attempt to relate these to any parental behaviour occurring.

7.2 Method

All males of pairs used in this experiment were vasectomized prior to the start of testing. Exposures were otherwise carried out as described in section 2.3. All adults received 3 PP exposures, then were given 1 UP exposure (See Figure 6.1). All animals which attacked pups had the UP exposures stopped and were given one further PP exposure. This was repeated for all adults until they were non-aggressive. Once they responded non-aggressively, each adult received a total of 10 UP exposures. If an animal reverted to an aggressive response after 2 or more UP exposures, that UP exposure was stopped. The animal received no further PP exposures, but was given further UP exposures, up to a total of 10. (See Figure 6.1).

In all exposures, ultrasonic vocalizations were recorded for the final three minutes of each ten minute exposure period. To record ultrasonics, a Bruel and Kjaer condenser type 4135 microphone was fixed in position above the stimulus pup. The microphone was connected to a Bruel and Kjaer Measuring amplifier, Type 2610. The amplifier and an oscillator were connected to an EMI SE 3000 Portable Instrumentation Recorder. (See Fig. 7.1). Tape speed was set at 191 mm per sec for recording. A double pulse of a 15kHz signal from the oscillator marked the beginning of the

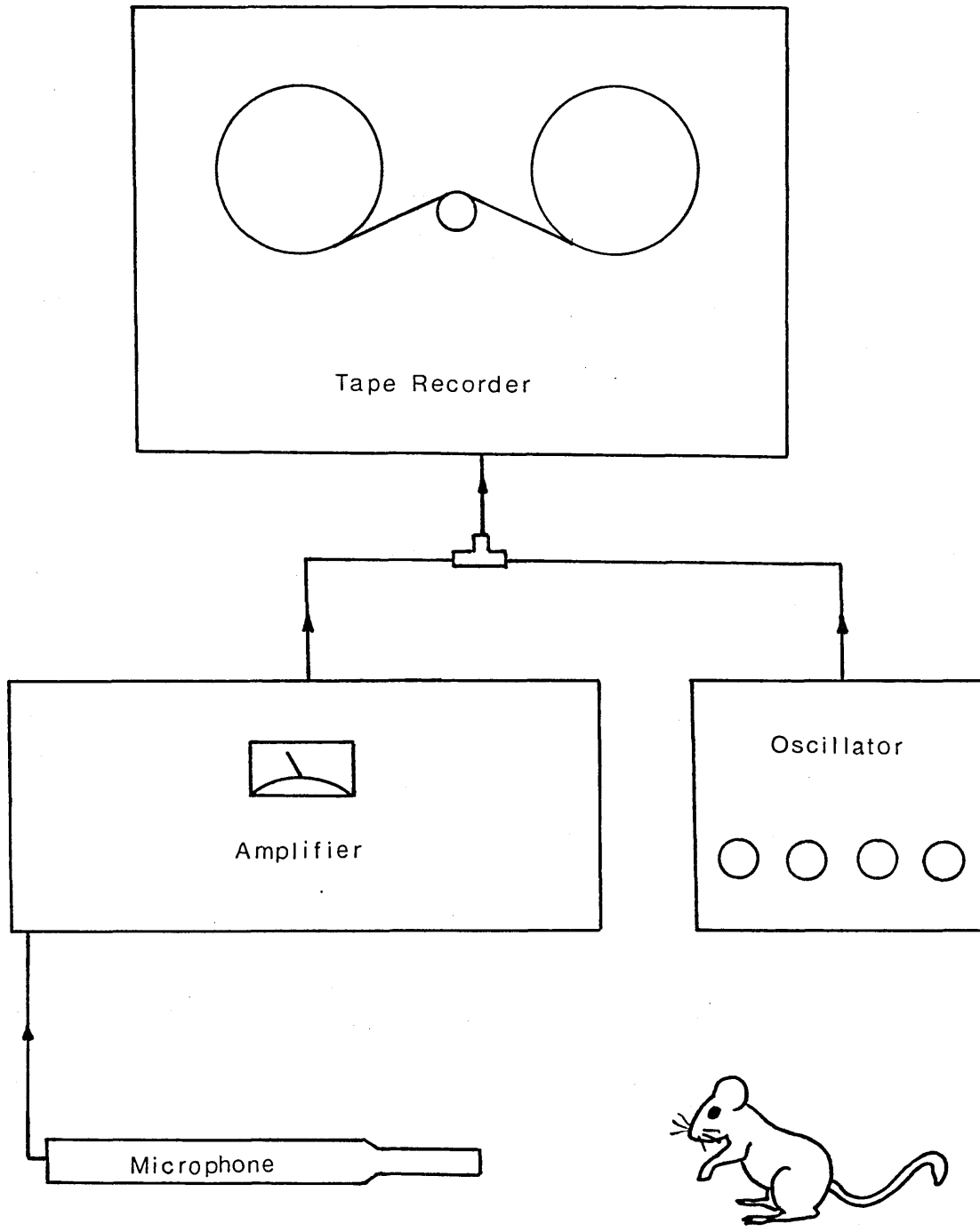

Figure 7.1 Recording ultrasonic vocalizations.

recording, and a single pulse marked the end. To listen to the vocalizations, recordings were played back at a quarter of the recording speed, that is 47.6mm per sec. which reduced the frequency of vocalizations by a factor of four, making them audible (Fig 7.2). The number of calls heard was tabulated alongside the behaviour occurring in the corresponding 15s interval (Figure 2.7).

Several calls were selected as being typical of those heard, ans were transferred to cassette tapes. These calls were then produced as sonagrams.

7.3 Results

Characteristics of individual calls were very variable. It was not possible to identify the source of calls as being either adults or pups. However, it was thought that most adult calls would be upsweeps, which are easily recognised when heard. Several were heard, but several other types of call were also heard. This suggests calls were from both the adults and the pups. Some calls on the sonagrams have been identified as pup calls due to the presence of audible components.

7.3 i Correlation of rate of calls with adult responses

No significant correlation was found between the total number of calls and the amounts of parental behaviour shown in the UP exposures for all animals (r_s = -0.13), or for males and females

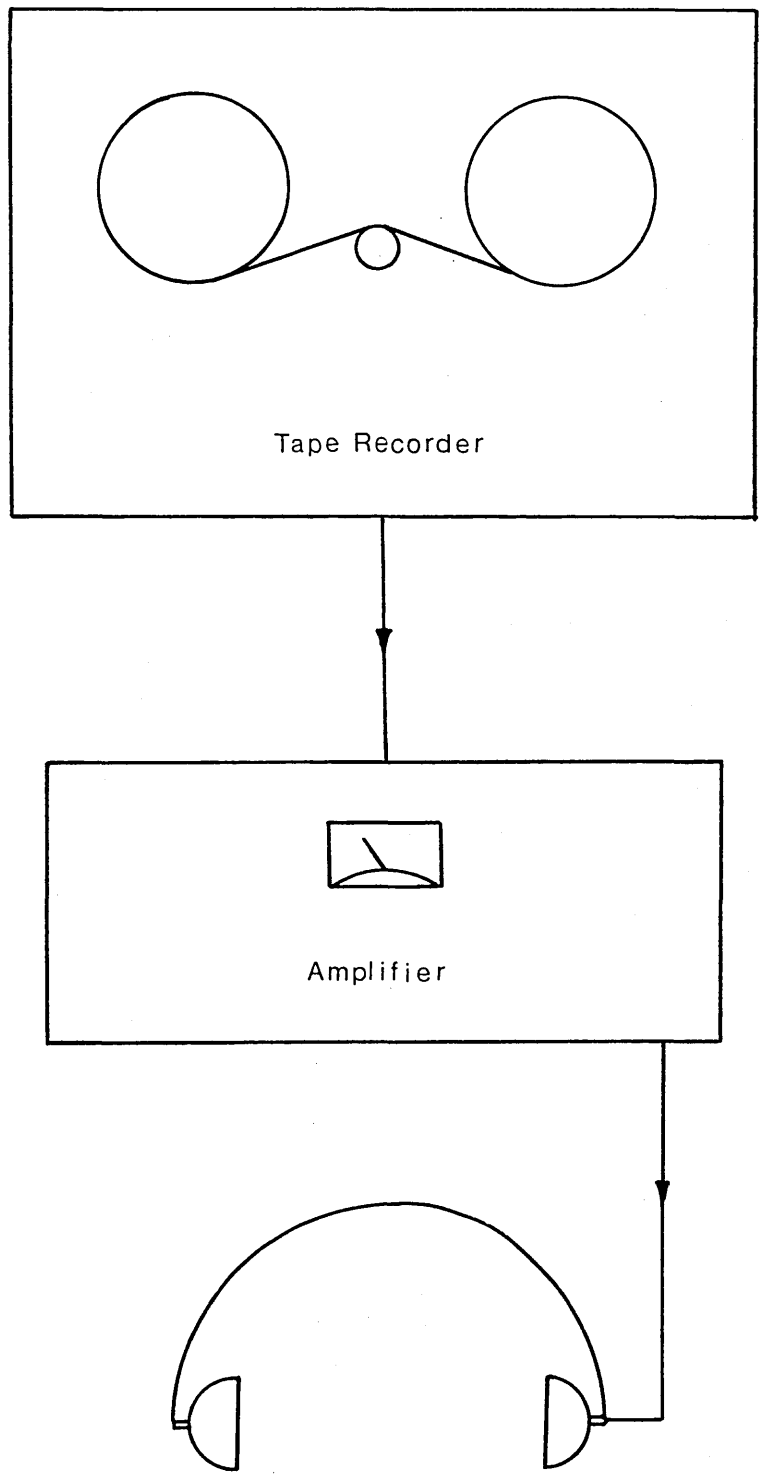

Figure 7.2 Playback of ultrasonic vocalizations.

singly (Table 7.1). No correlation was found between the numbers of PP exposures needed before a non-aggressive response was given in a UP exposure and the rate of calling in the PP exposures for all animals (r_s = 0.01), or for males and females singly. (Table 7.2).

When results of correlations of ultrasound with behaviour and numbers of PP exposures were analyzed separately for the two sexes, differences were found. The correlation between the rate of ultrasonic calling and the frequency of parental behaviour patterns shown was insignificant for males (r_s = 0.06). Although females showed the best negative correlation (r_s = -0.36) between increased rate of calling with decreased parental behaviour, the correlation was not significant. There was little difference between males and females for r_s for rate of calling and PPs required to produce a non-aggressive response ($\sigma r_s = 0; \varphi r_s = 0.04$). For males this appears to be totally independent of rate of calling.

7.3 ii Sonagrams

Examples of sonagrams of typical calls recorded are shown in figures 7.3 - 7.8. Figure 7.3 shows a single pup call with both ultrasonic (above 16 kHz) and audible (below 16 kHz) components. Figure 7.4 shows two pup calls similar in structure, and occurring in quick succession. Again these show ultrasonic and audible components. Figure 7.5 shows two pup calls ocurring in quick succession. Ultrasonic and audible components are seen, but with

Table 7.1 Median values of frequencies of parental behaviours[Δ] and rate of ultrasonic calling.

Pair	♂♂		♀♀	
	Frequency	Calls per min.	Frequency	Calls per min.
1	15.0	10.0	7.5	6.0
2	13.5	8.0	14.5	7.0
3	9.5	13.0	12.0	2.0
4	13.5	13.5	6.0	16.0
5	9.0	6.5	4.0	6.0
6	13.5	4.0	10.0	20.0
7	12.0	12.5	12.5	4.0
8	16.5	2.5	12.0	18.0
9	19.0	14.0	15.0	3.5
10	19.0	9.5	12.0	6.0
r_S (male and female combined)		-0.13		
r_S*		0.06		-0.36

Δ parental behaviours = investigate pup, sit over pup, nest-build, retrieve, arch back.

* Spearman rank correlation coefficient.

Table 7.2 Median rate of calls per minute in PP exposures and numbers of PP exposures required to produce a non-aggressive response in UP exposures.

Pair	PPs ♂♂	Calls per min.	PPs ♀♀	Calls per min.
1	3	74.0	5	0
2	3	15.0	4	25.0
3	3	17.0	3	10.0
4	3	62.0	3	9.0
5	3	11.0	4	7.5
6	3	3.0	6	33.0
7	3	36.0	3	15.0
8	3	3.0	5	3.0
9	3	6.0	3	1.0
10	3	4.0	4	37.0
r_S (male and female combined)		0.01		
r_S *		0		0.04

* Spearman rank correlation co-efficient.

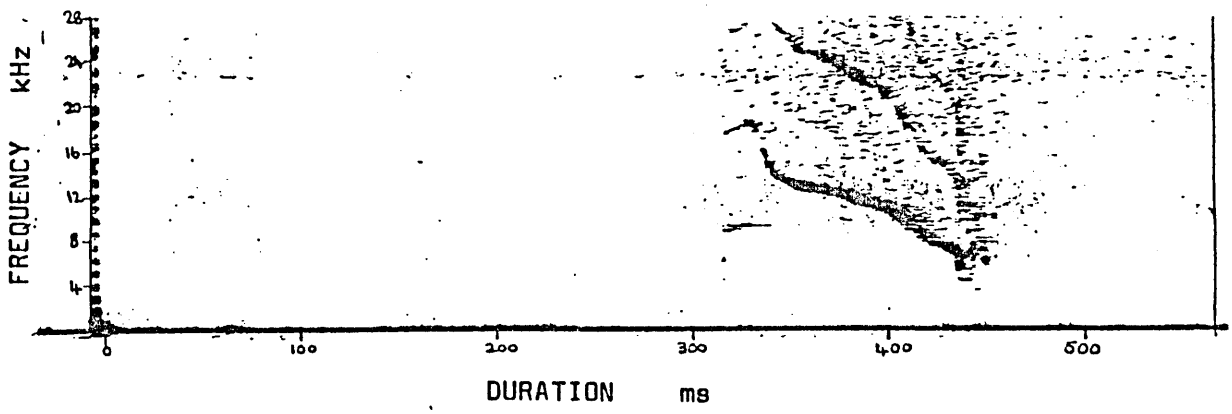

Figure 7.3 Single pup call.

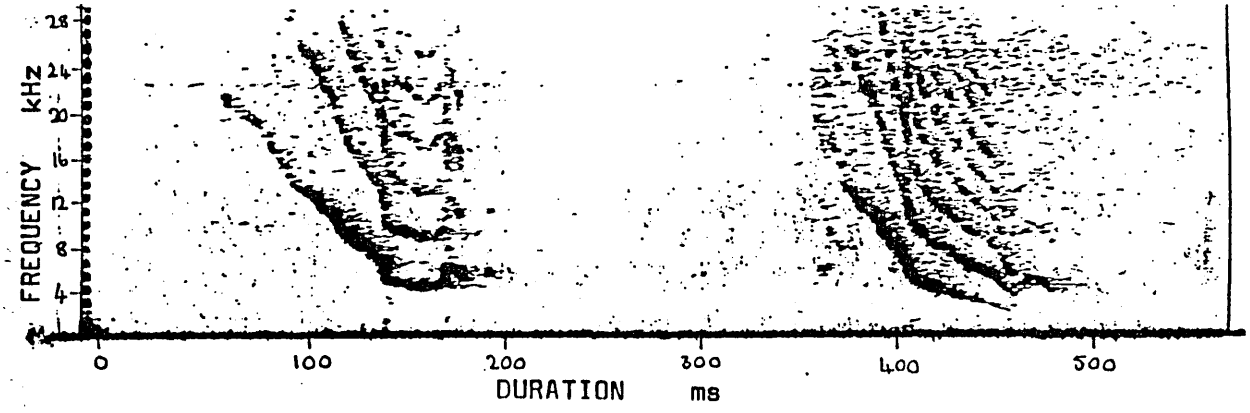

Figure 7.4 Two pup calls.

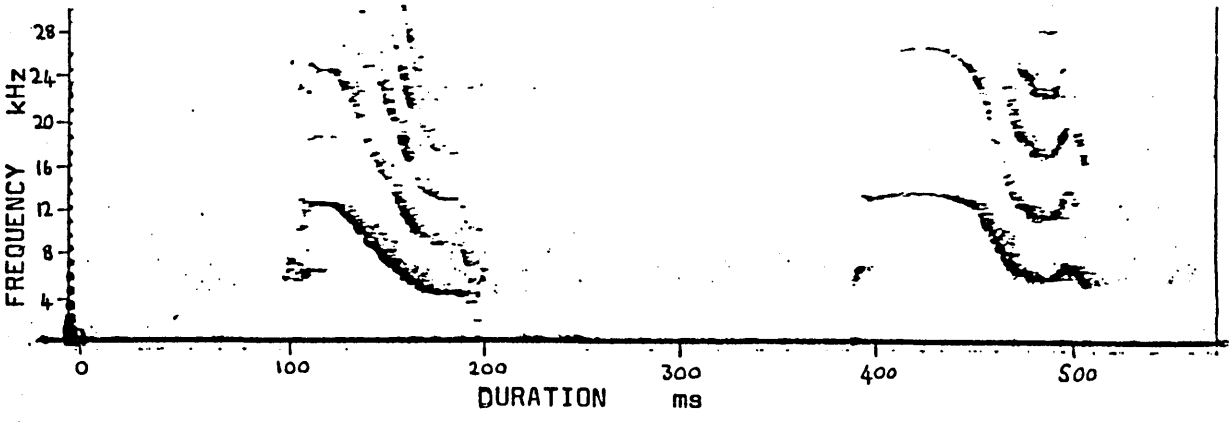

Figure 7.5 Two pup calls.

fewer harmonics than in figure 7.4. Figure 7.6 shows a pure ultrasonic call (a), followed by an ultrasonic/audible pup call (b), followed by an ultrasonic upsweep (c). The two pure ultrasounds may have come from either the adult or the pup. Figure 7.7 shows a modulated ultrasonic call (a) with an audible call (b). The ultrasonic call may have come from the adult or the pup. Figure 7.8 shows an unmodulated ultrasonic call, which may have come from the adult or the pup.

Summary
1.No significant correlation was found between the rate of ultrasonic calling and either the number of PP exposures required to produce a non-aggressive response or the frequency of parental behaviour patterns. However, there were sex-differences in the results.
2. For males the number of PP exposures required to produce a non-aggressive response is independent of the rate of ultrasonic calling. The frequency of parental behaviours is very slightly but insignificantly correlated with the rate of ultrasonic calling in a positive direction.
3. For females the number of PP exposures required to produce a non-aggressive response is very slightly correlated with the rate of ultrasonic calling in a positive direction. The frequency of parental behaviours is insignificant, but negatively correlated with the rate of ultrasonic calling.

7.4 Discussion.

7.4 i Correlation of rate of calls with adult responses

Sex differences between correlations of rate of ultrasonic calling and both the number of PP exposures required to produce a non-aggressive response in UP exposures and the frequency of parental behaviours suggest ultrasonic calls might influence the sexes in different ways. However from these results it seems calls have little influence on males and females.

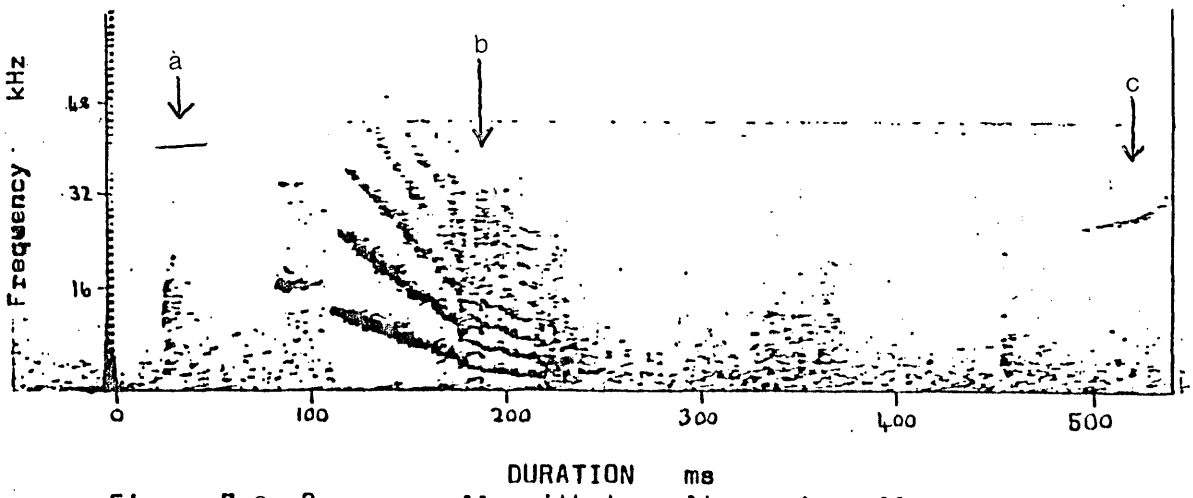

Figure 7.6 One pup call, with two ultrasonic calls, source not identified.

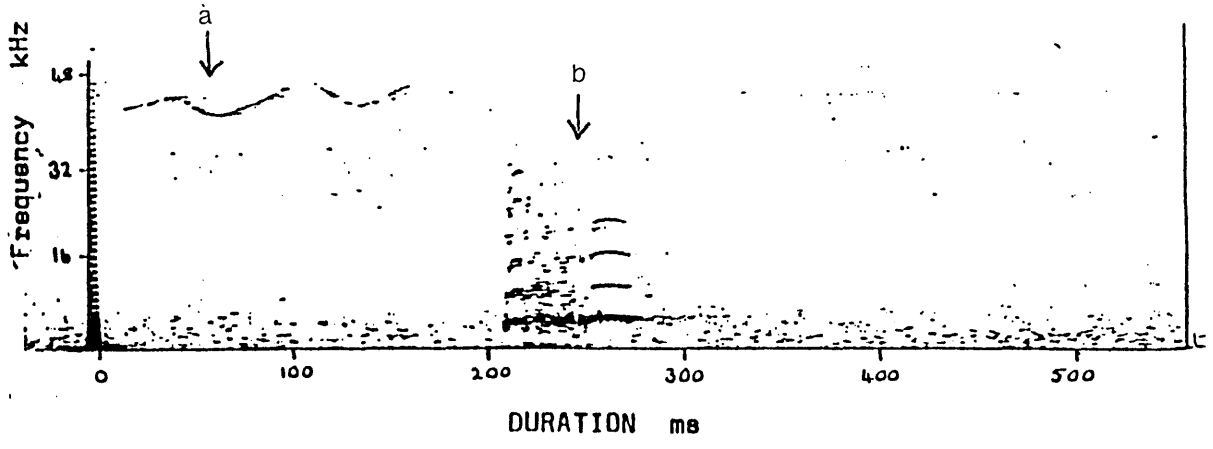

Figure 7.7 Two ultrasonic calls, source not identified.

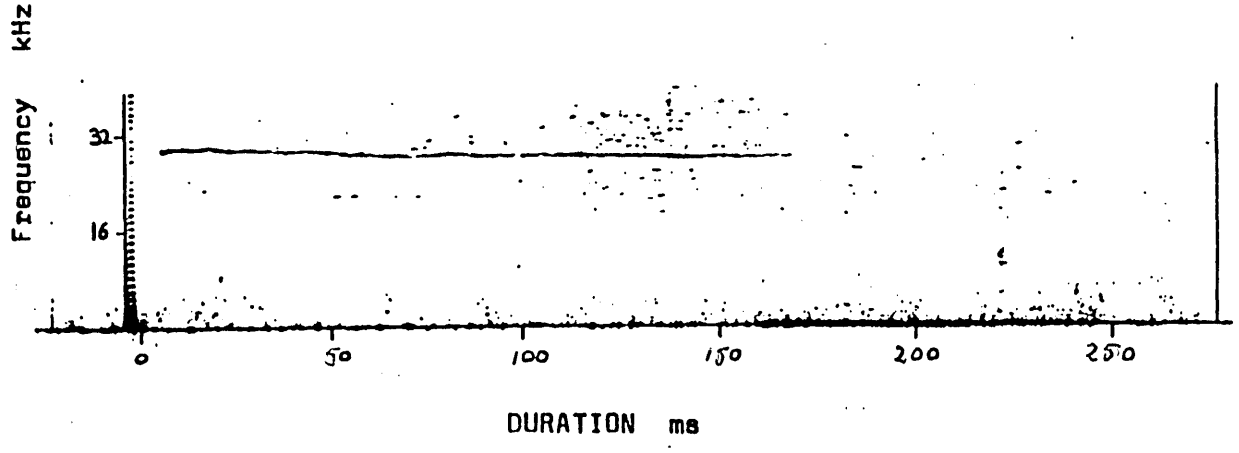

Figure 7.8 One ultrasonic call, source unidentified.

Several authors who have carried out work to demonstrate relationships between the ultrasonic calling of pups and its effect on parental behaviour have shown a correlation. Zippelius and Schleidt (1956) showed scattered pups would be retrieved by their mothers as long as the pups were able to call. If pups were unable to call, either when dead or narcotized, then they were not retrieved. Deaf 'fidget' mice have been shown to be very bad mothers, with their pups dying early in life, often scattered over the cage (Noirot and Pye, 1969).

Ultrasonic calls by mouse pups are thought to have a variety of functions. They may guide the adults towards the pups to allow the pups to be retrieved to the nest. A different type of call may also have an effect on nest-building by the adults when the pups are cold, and yet another type may inhibit aggression in the adult (Noirot, 1966a, 1972a). Calls have been shown to be emitted in response to cold in several rodent species. Calls affected by temperature cease with the gradual development of homiothermy as the pups grow older (Okon, 1970, 1971). This same phenomenon has been noted for gerbils (De Ghett, 1974) and rats (Allin and Banks, 1971).

From these and other earlier studies, it has been shown that the rate of ultrasonic calling by infant rodents, including Mongolian gerbils (Broom et al, 1977) is related to changes in maternal behaviour, and it was therefore expected that some correlation would have been found between rate of calling and amounts of parental behaviour produced in the work presented here. However, there were several aspects of the method used in this study which could account for no correlation being found. It is possible that the calls heard (Figs 7.3 - 7.5) are similar in effect to those noted by Noirot as being produced in response to discomfort, thus causing the withdrawal of the adult, and cessation of its rough handling of the pup or aggression (see also

Elwood and McCauley, 1983). It may be that the occurrence of these calls inhibited the adults' parental behaviour.

Another aspect of the method used relates to the calls which were produced in response to pup cooling (Noirot, 1972a). The temperature in the observation chamber was noted as being 33 - 34.5°C, which is higher than temperatures noted by Noirot as inducing calling. It may be that the environmental temperature was sufficient to keep the pups warm in the absence of their mother, thus not inducing similar rates of calling as those found to be related to maternal behaviour. However it was frequently noted that pups felt cool to the touch at the end of a ten minute exposure, so that it is unlikely this type of call was stopped altogether.

A further aspect of the study which may account for no significant correlation being found between rate of calling and parental behaviour is that the source of calls was not identified, and may have been a mixture of pup and asdult calls. Adult Apodemus sylvaticus were observed to produce ultrasonic pulses in response to cage disturbance (Sewell, 1968). It is also a possibility that adults produced ultrasonic calls similar to those emitted during aggressive encounters in other rodent species (Sales, 1972). In an attempt to clarify this, recordings were made of solitary pups and adults in experimental conditions (that is, only the pup or the adult was present). Only one call was recorded from the adults, whereas pup calling was frequent and repetitive as in earlier pup/adult recordings. It therefore seems reasonable to conclude the influence of adult calls was negligible, and that they did not account for the lack of correlation found between call rate and behaviour.

As already mentioned, Ralls (1967) and Brown (1973a,b) have shown that adults will in fact be able to hear the calls of pups,

with their peak hearing corresponding to the call frequency of their specific young.

From the results of similar work with other rodents, and in considering the findings of Broom et al (1977), it would be expected that on further investigation of gerbil pup calling, different types of call could be detected, and these in turn might affect different aspects of parental behaviour.

7.4 ii Sonagrams

The pup calls seen in figures 7.3 - 7.5 are possibly calls produced in response to discomfort due to the adult handling the pup, since frequently audible calls were heard when pups were picked up or licked by the adults. Figures 7.6 and 7.7 show different types of call, possibly all coming from the pup, or perhaps some from the pup and some from the adult. The upsweep, modulated and unmodulated calls are characteristic of adult calls (see Holman and Hutchison, 1985), suggesting these may indeed be from the adult. As discussed earlier, this seems extremely unlikely, and it can be concluded that all calls were from pups.

CHAPTER EIGHT MAINTENANCE OF PARENTAL RESPONSIVENESS.

8.1 Introduction

As parental responsiveness similar to that shown by both male and female parent gerbils after the birth of a litter had been produced in naive males and females, this experiment was aimed at seeing if the pup-exposure induced responsiveness was as permanent as the natural parental behaviour. Several rodent species have been studied to see how long maternal responsiveness would be maintained after parturition. In rats, some females retained their responsiveness to pups up to 186 days post parturition, while others were responsive up to 400 plus days post parturition (Wiesner and Sheard, 1933).This extended responsiveness was obtained by constantly replacing the pups with others of a younger age, removing the older ones at this time so that only one litter was present at any one time. The overall effect was as if pups were not maturing. This meant that stimuli from the pups were constantly the same as those from a very young litter. Normally the maternal responsiveness would have disappeared gradually, with times spent in various behaviour patterns declining to very low levels by day 21 post partum. (Grota and Ader 1969).

In hamsters, females will usually look after their pups when they are born, but their aggression to pups will return when lactation finishes. The animals will remain aggressive to pups

almost until another litter is born (Rowell, 1961b).

With gerbils, it has been reported that females lose their aggression towards pups in late pregnancy, and regain aggression to new-born and therefore strange pups 11-25 days post partum. Males lose their aggression when their mate is approximately 7-12 days pregnant, and remain non-aggressive if kept housed with their pregnant mate. Once they have had one experience of raising a litter, males will never revert to being aggressive towards pups (Elwood 1980,1981).

In view of this, a procedure was planned to test for the maintenance of any non-aggressive and parental responses shown to pups by naive adults which had been exposed to pups in three of the experiments (those earlier described in Chapters 4, 5 and 6).

8.2 Method

Three separate groups of animals were tested for maintenance of a non-aggressive response to pups. Subjects used in this experiment were those used in experiments described in Chapters 4, 5 and 6. (Chapter 4 animals were housed in large cages, Chapter 5 animals were housed in small cages and Chapter 6 animals had their odour altered. Only animals from the 'sebum' experiment were considered here). The numbers of pairs making up Groups 1 and 2 were less than those described in Chapters 4 and 5 as one member of each of seven pairs (4 from Gp 2 and 3 from Gp 1) had died. (See Table 8.1 for testing regime).

Table 8.1 Testing regime for maintenance experiments.

	UP EXPOSURES		
	ORIGINAL	2 WK M	10 WK M
Group 1	5 PP,5 C,1 UP	3 PP,2 UP	2 PP,4 UP
Group 2	up to 18 PP,3 UP	3 UP	2 UP
Group 3	up to 8 PP, 10 UP	5 UP	5 UP

2wk M = 2 week maintenance

10wk M = 10 week maintenance

C = control exposure

8.2 i Maintenance periods

Two periods were selected to be tested for maintenance. In the normal laboratory situation, females are known to maintain their responsiveness to pups as long as they are suckling, and males are known never to lose their responsiveness once they have experienced raising a litter. For this reason 2 weeks (2wk M) and 10 weeks (10wk M) after original UP exposures were used to coincide with times which would be during lactation, and well after the end of lactation.

8.2 ii Group 1

Approximately two weeks after the single UP exposure, five pairs of the animals received three PP exposures and 2 UP exposures. Approximately 10 weeks after the initial UP exposure, the 5 pairs received 2 PP exposures followed by 4 UP exposures.

8.2 iii Group 2

Approximately 2 weeks after the original 3 UP exposures, 9 pairs received a further 3 UP exposures. Approximately 10 weeks after the original 3 UP exposures, 5 pairs of animals received a further 2 UP exposures.

8.2 iv Group 3

Approximately two weeks after the original 10 UP exposures, all animals (10 pairs) received 5 UP exposures. Approximately 10 weeks after the original UP exposures, all animals received a further 5 UP exposures. In this particular experiment, the pups were again rubbed on the experimental adult's scent gland as described in section 6.2.

8.3 Results.

8.3 i Aggression

Table 8.2 shows numbers of animals showing an aggressive response to an unprotected pup on at least one occassion during a series of UP exposures. Group one males showed a decrease in the number of animals showing an aggressive response from original to 2wk M exposures, followed by a slight increase from 2 to 10wk M exposures. Group 2 males showed a similar response, while Group 3 males showed an increase in the number of animals showing an aggressive response at each time interval. In all groups, females showed an increase in numbers of animals showing an aggressive response on at least one occassion during a series of UP exposures, both from original to 2wk M, and from 2 to 10wk M exposures.

Table 8.3 shows numbers of aggressive responses shown to pups by males and females in Groups 1, 2 and 3 in each series of UP exposures. In Group 1, males showed a decrease in levels of aggressive responses from original to 2wk M UP

Table 8.2 Numbers of animals showing an aggressive response on at least one occasion in a series of UP exposures.

Group	UP exposure series			p*		
	0wk ■	2wk ○	10wk □	■ ○	■ □	○ □
1 ♂	5/8	2/6	3/5			
2 ♂	4/9	3/9	2/5			
3 ♂	0/10	2/10	7/10		<0.003	
1 ♀	5/8	4/6	5/5			
2 ♀	7/9	8/9	5/5			
3 ♀	3/10	6/10	8/10			

* Fisher exact probability test.

Fractions - numerator = number of animals responding aggressively on at least one occasion in a series of UP exposures

denominator = number of animals tested

Table 8.3 Number of aggressive responses during series of UP exposures.

Group	UP exposure series			p*		
	■ 0wk	○ 2wk	□ 10wk	■ ○	■ □	○ □
1♂	5/8	3/12	5/20			
2♂	10/27	9/27	3/10			
3♂	0/100	6/50	13/45	<0.001	<0.001	
1♀	5/8	8/12	15/20			
2♀	17/27	20/27	8/10			
3♀	13/1000	23/50	24/45	<0.001	<0.001	

* Fisher exact probability test

Fractions - numerator = total number of aggressive responses shown during UP exposures

denominator = total number of UP exposures

exposures, then a slight increase from 2 to 10 wk M exposures. Females showed no change in levels of aggressive responses over time. In Group 2, males showed little change, and females slight increases in levels of aggression over time. In Group 3, males showed a slight increase in levels of aggressive responses from original to 2wk M exposures. This increase was significantly different between original and 2wk M exposures ($p < 0.001$ Fisher exact probability test) and between original and 10wk M exposures ($p < 0.001$). Group 3 females showed an increase in levels of aggressive responses from original to 2wk M exposures, then a slight increase from 2wk to 10wk M exposures. The difference between original and 2wk M exposures was significant ($p < 0.001$), as was the difference between original and 10wk M exposures ($p < 0.001$).

8.3 ii Parental patterns

Figures 8.1 - 8.14 show the median frequencies of behaviour patterns shown by non-aggressive males and females in Groups 1, 2 and 3 in UP M exposures (where no value is shown the behaviour pattern was not shown, as opposed to a median value of zero which is recorded on the graphs). Group 2 males showed a significant decrease in time spent sniffing the pup (Fig. 8.5) from original to 2wk M UP exposures ($T = 0$, $p < 0.05$). Group 3 males showed a significant increase in time spent sniffing their own cage (Fig. 8.4) from original to 2wk M exposures ($T = 5.5$, $p < 0.05$), in time spent sitting over the pup (Fig. 8.8) from original to 2wk M UP exposures ($T = 4$, $p < 0.02$), and in time spent sitting over the

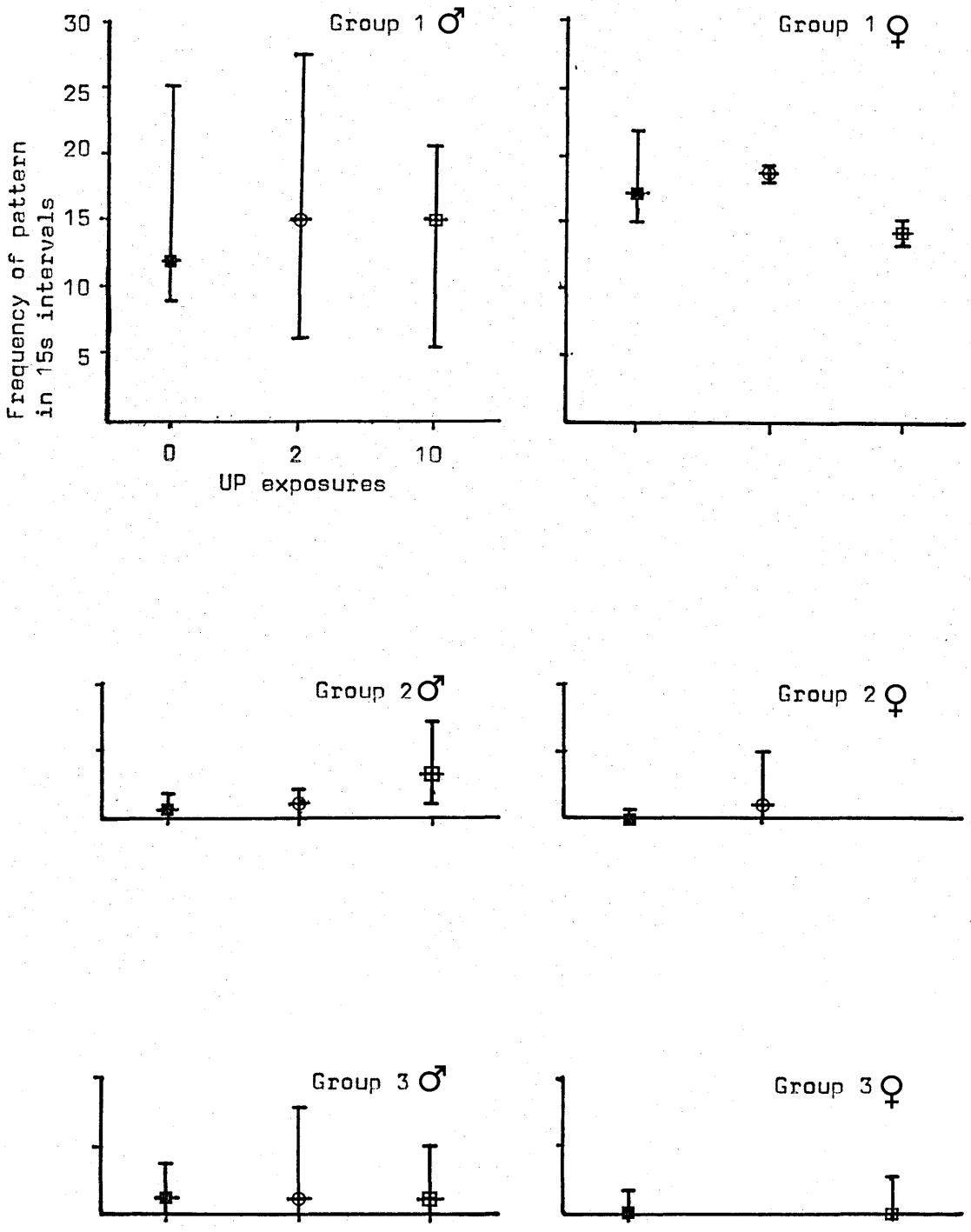

Figure 8.1 Inactive

The positions of the graphs are as follows: top, Group 1; middle, Group 2; bottom, Group 3. Males, left hand graphs; females, right hand graphs.
Abscissa represents the time intervals of original (■), and the following 2 (O) and 10 (□) week maintenance UP exposures. Ordinate represents the median frequency of pattern (plus ranges) in 15s intervals.
Differences between the medians, when significant, are shown in tabular form.

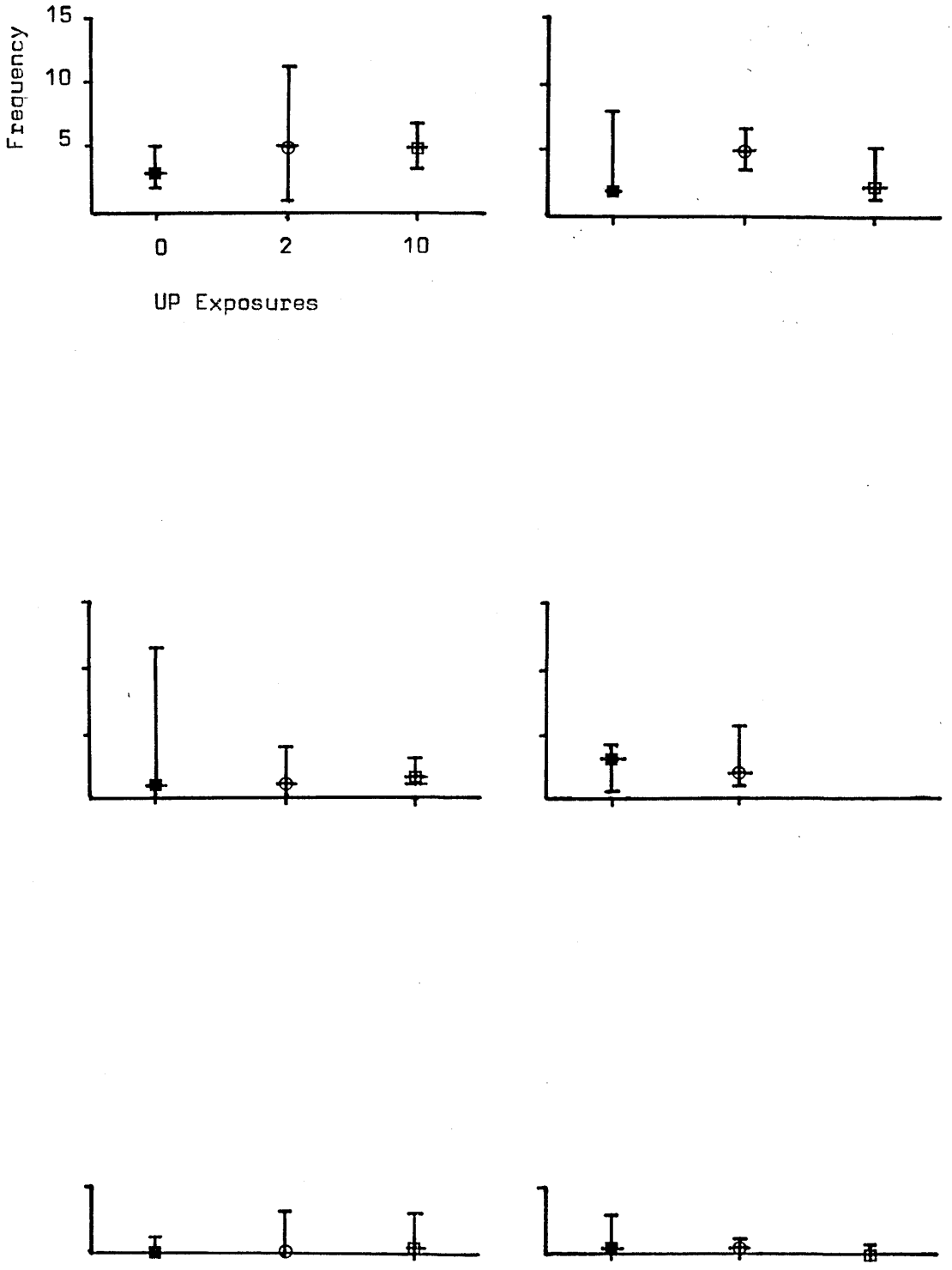

Figure 8.2 Groom self
(Legend as for figure 8.1)

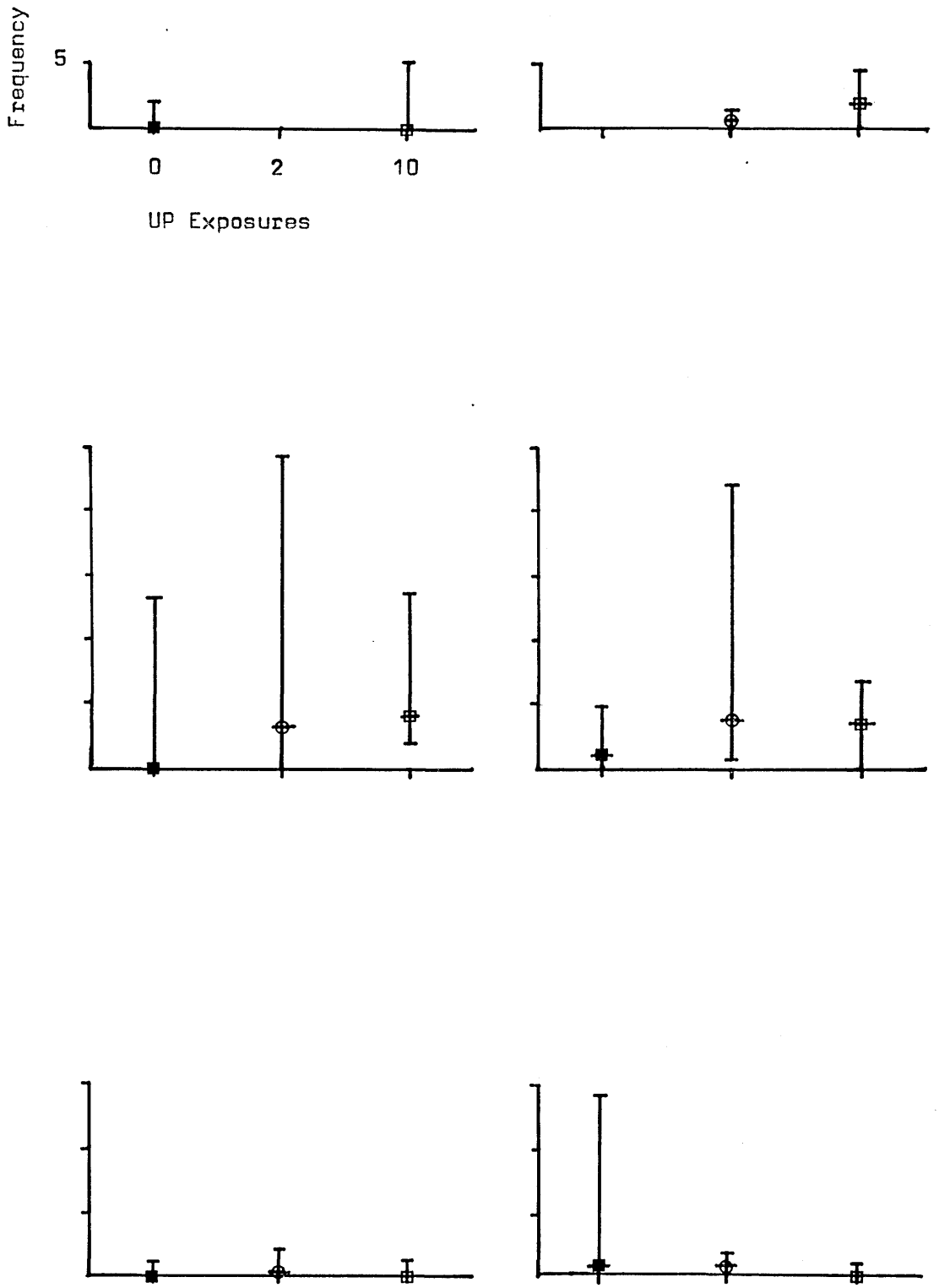

Figure 8.3 Scratch cage
(Legend as for figure 8.1)

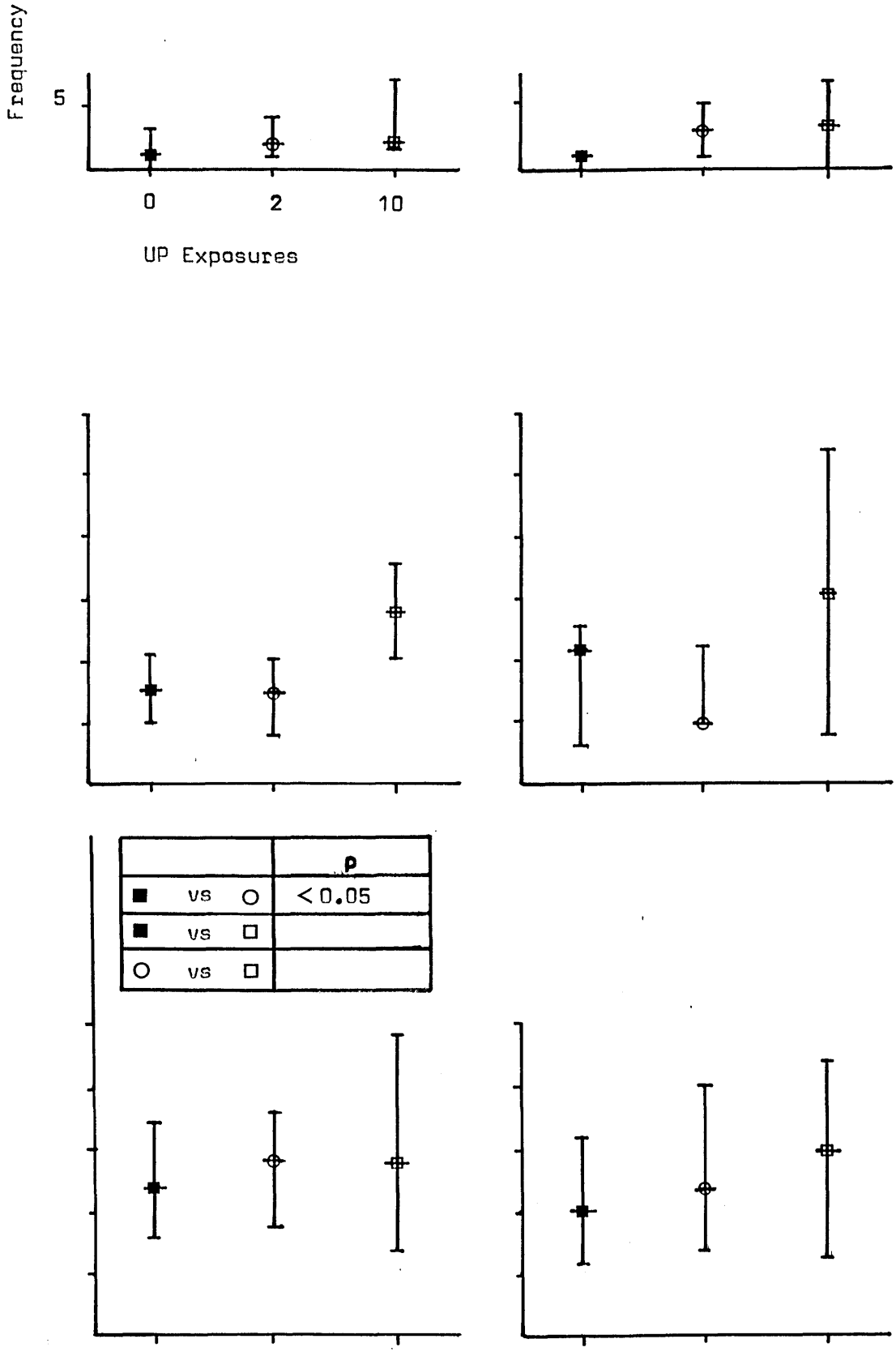

Figure 8.4 Sniff own cage
(Legend as for figure 8.1)

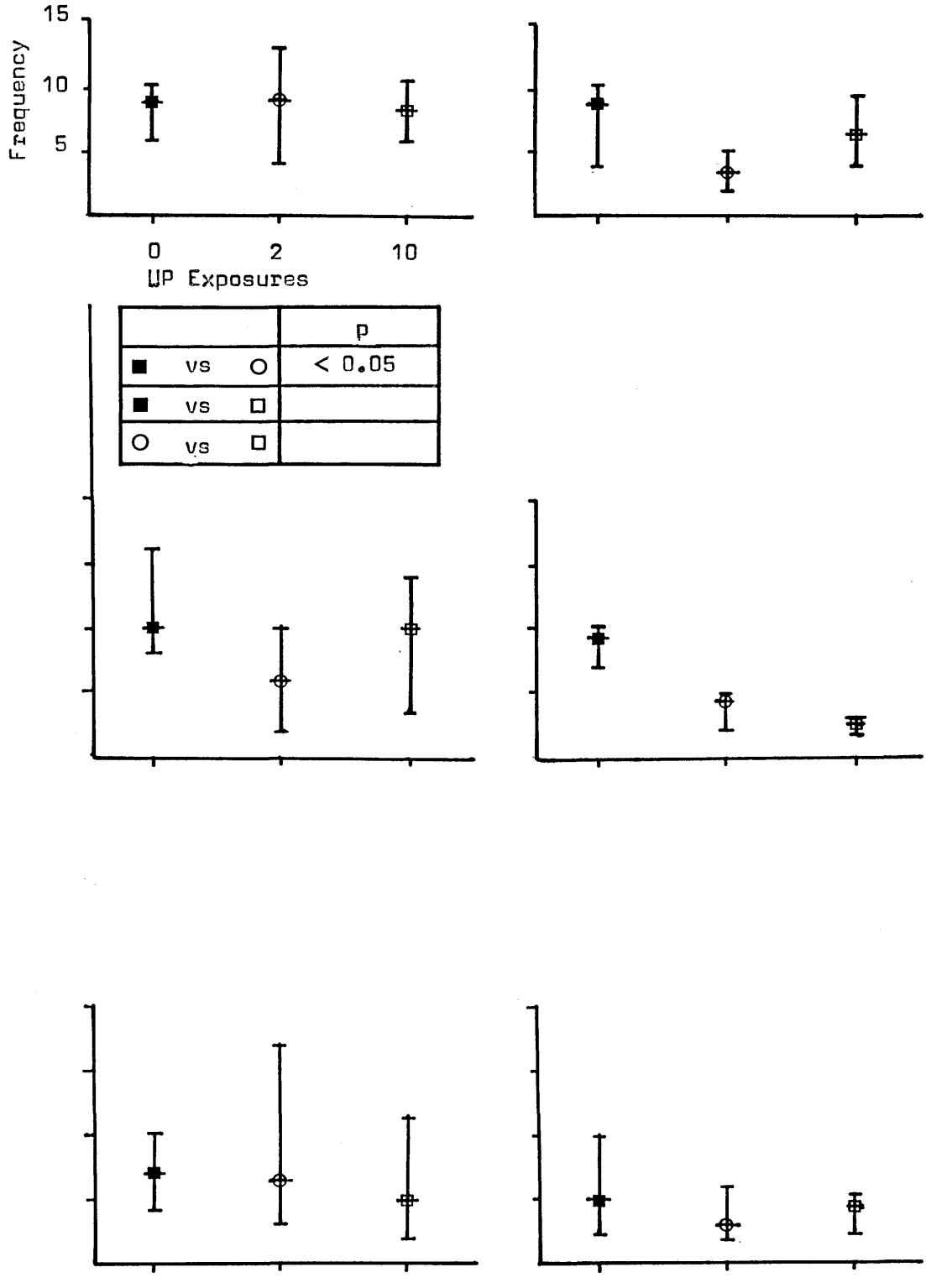

Figure 8.5 Sniff pup
(Legend as for figure 8.1)

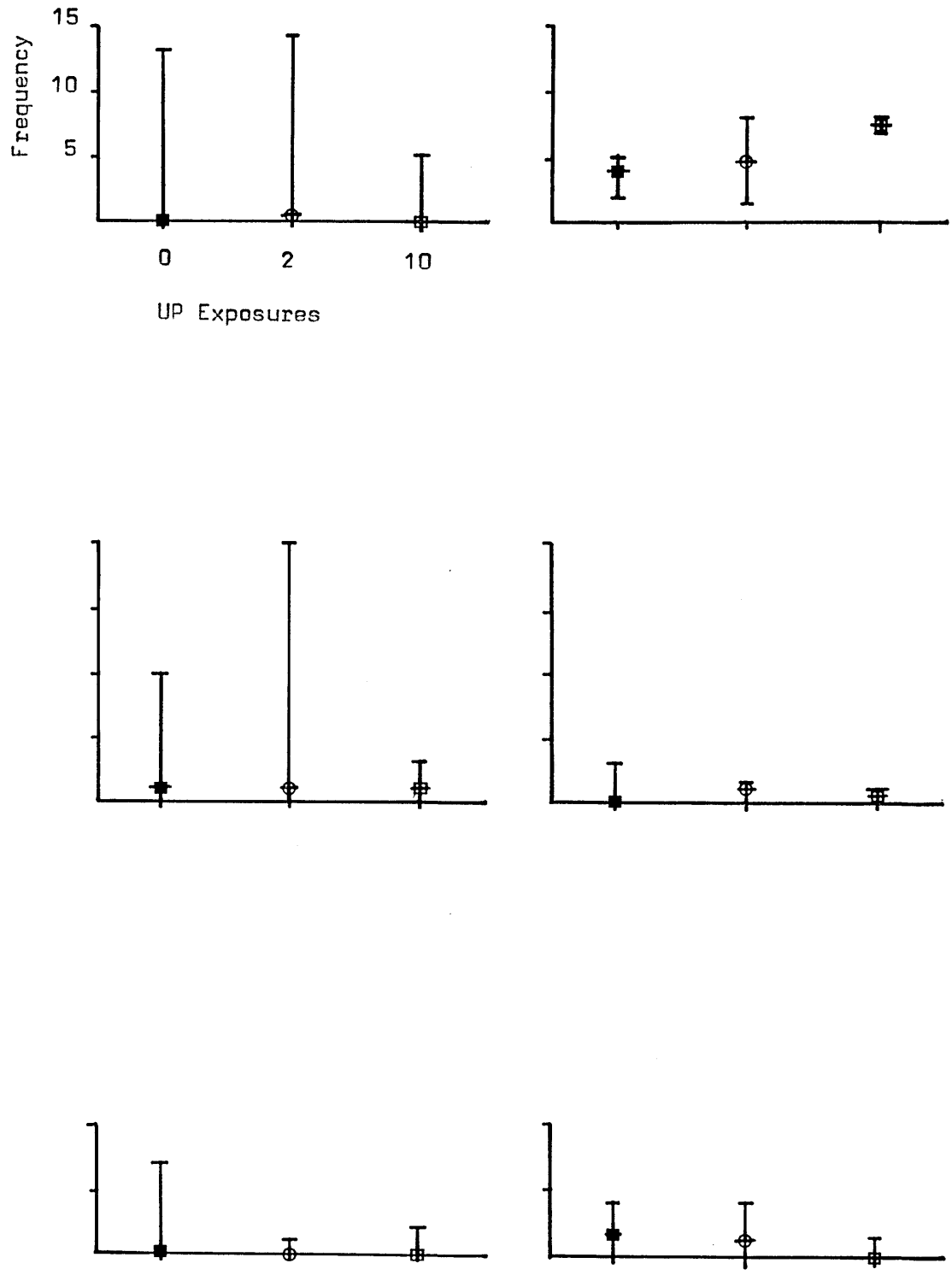

Figure 8.6 Tear paper
(Legend as for figure 8.1)

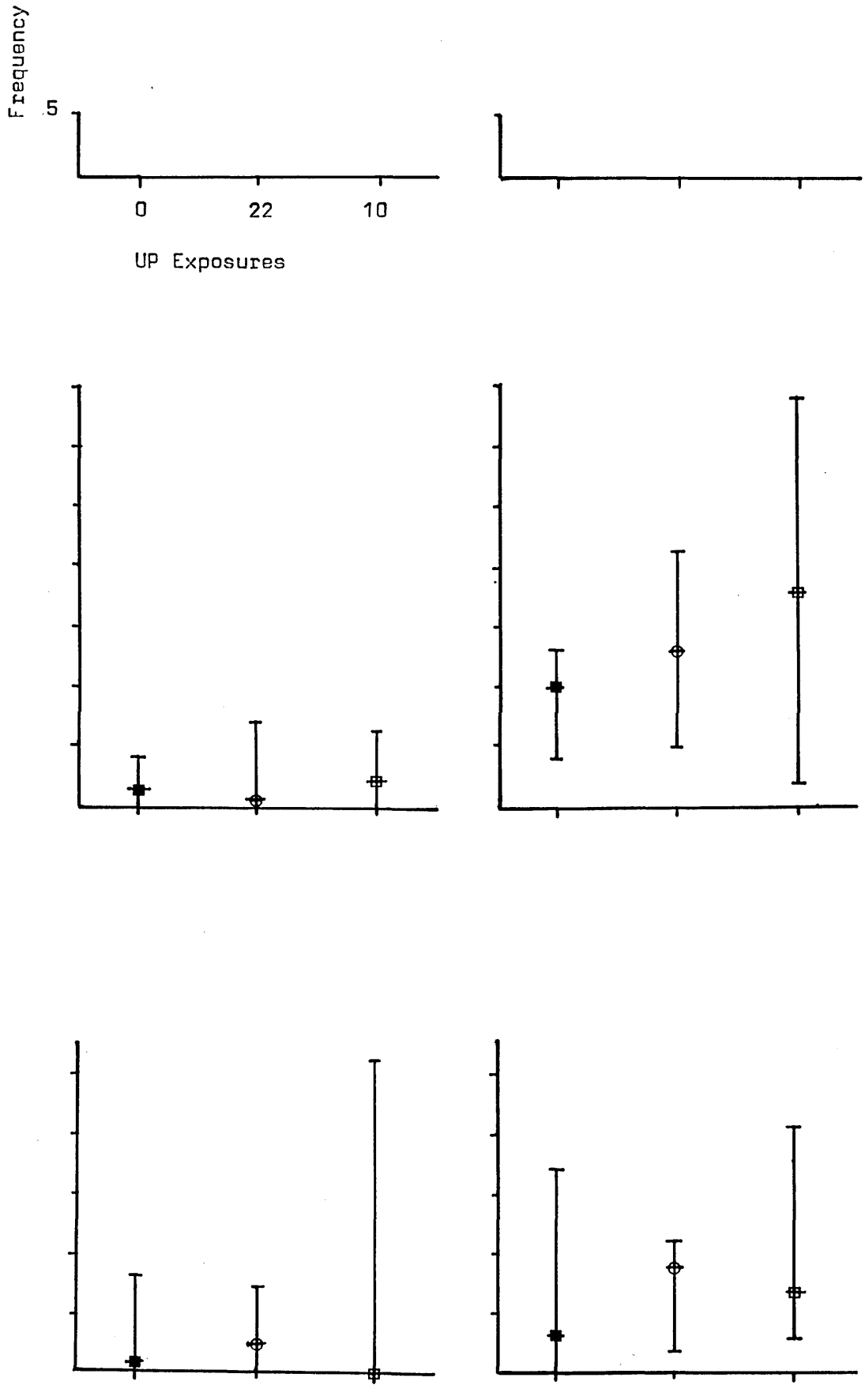

Figure 8.7 Burrow

(Legend as for figure 8.1)

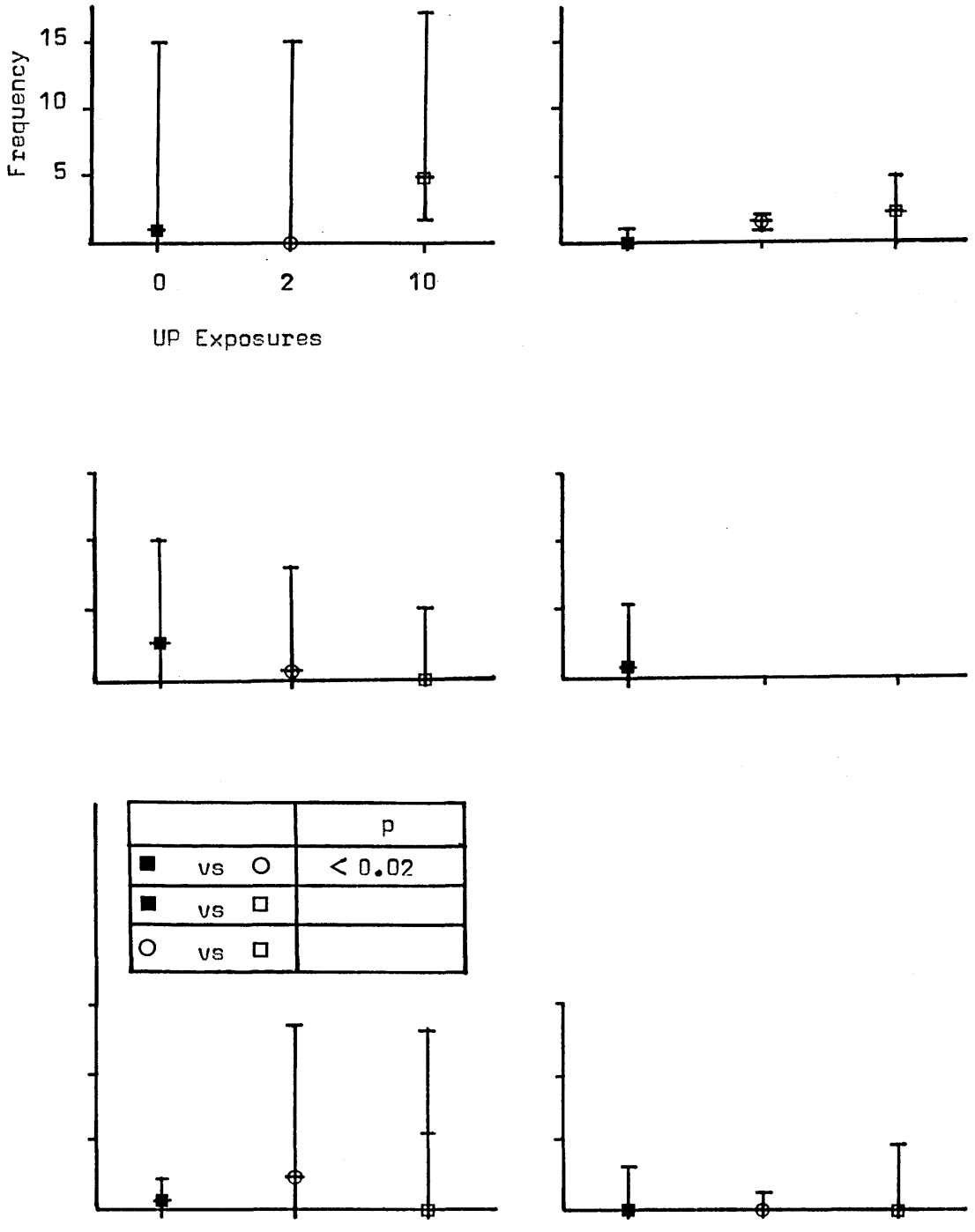

Figure 8.8 Sit over

(Legend as for figure 8.1)

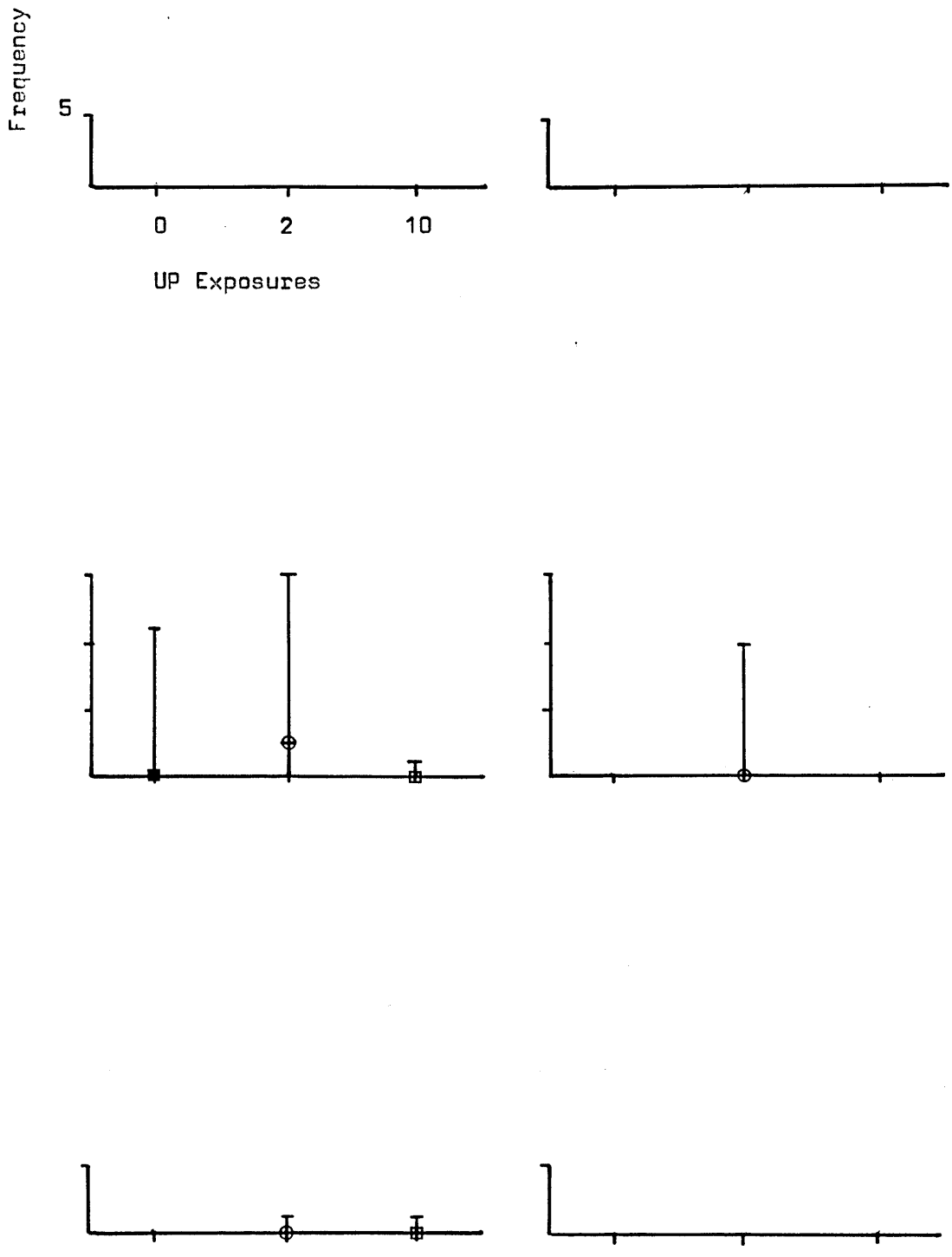

Figure 8.9 Gnaw own cage

(Legend as for figure 8.1)

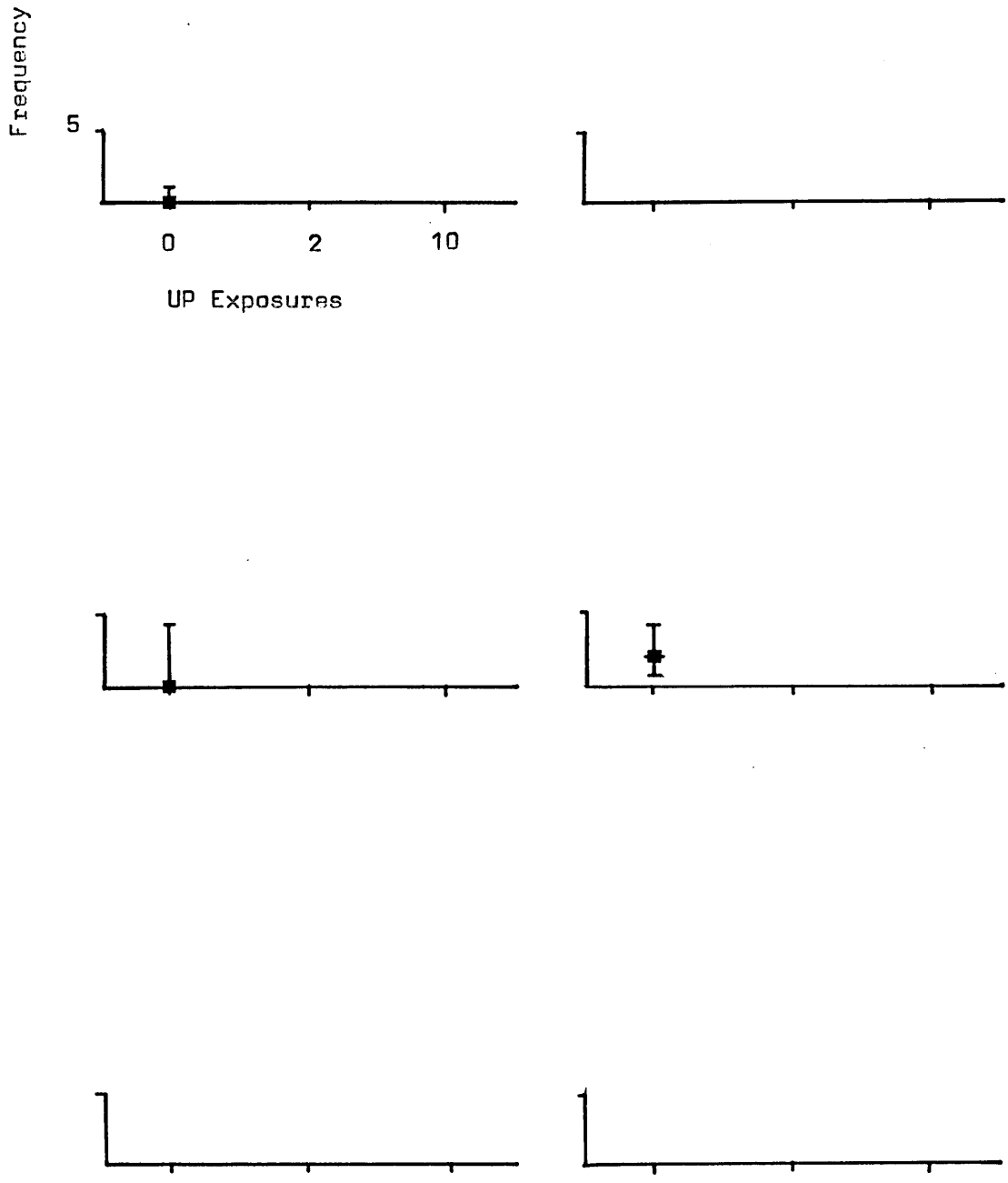

Figure 8.10 Bury pup
(Legend as for figure 8.1)

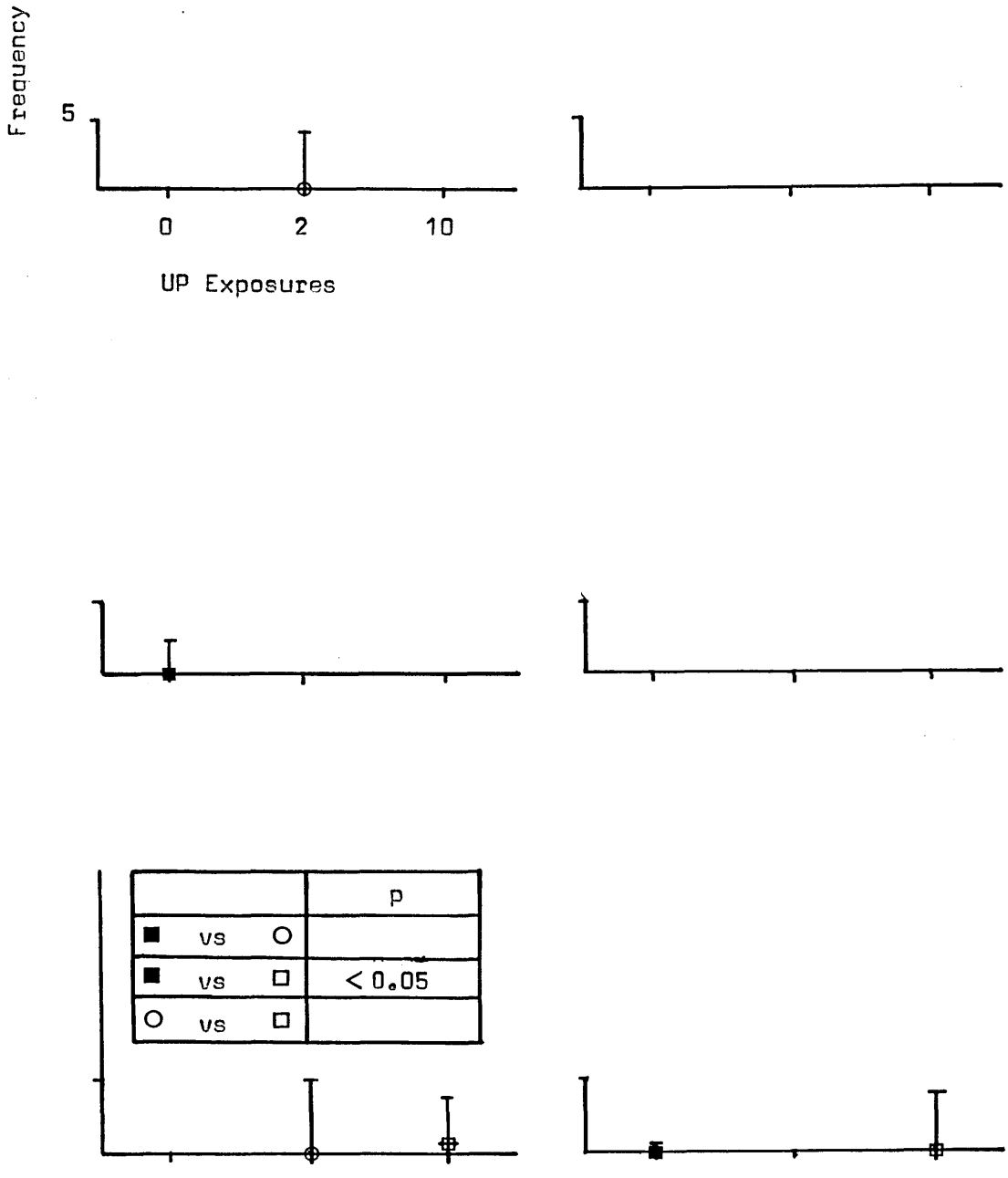

Figure 8.11 Sit over and nest-build

(Legend as for figure 8.1)

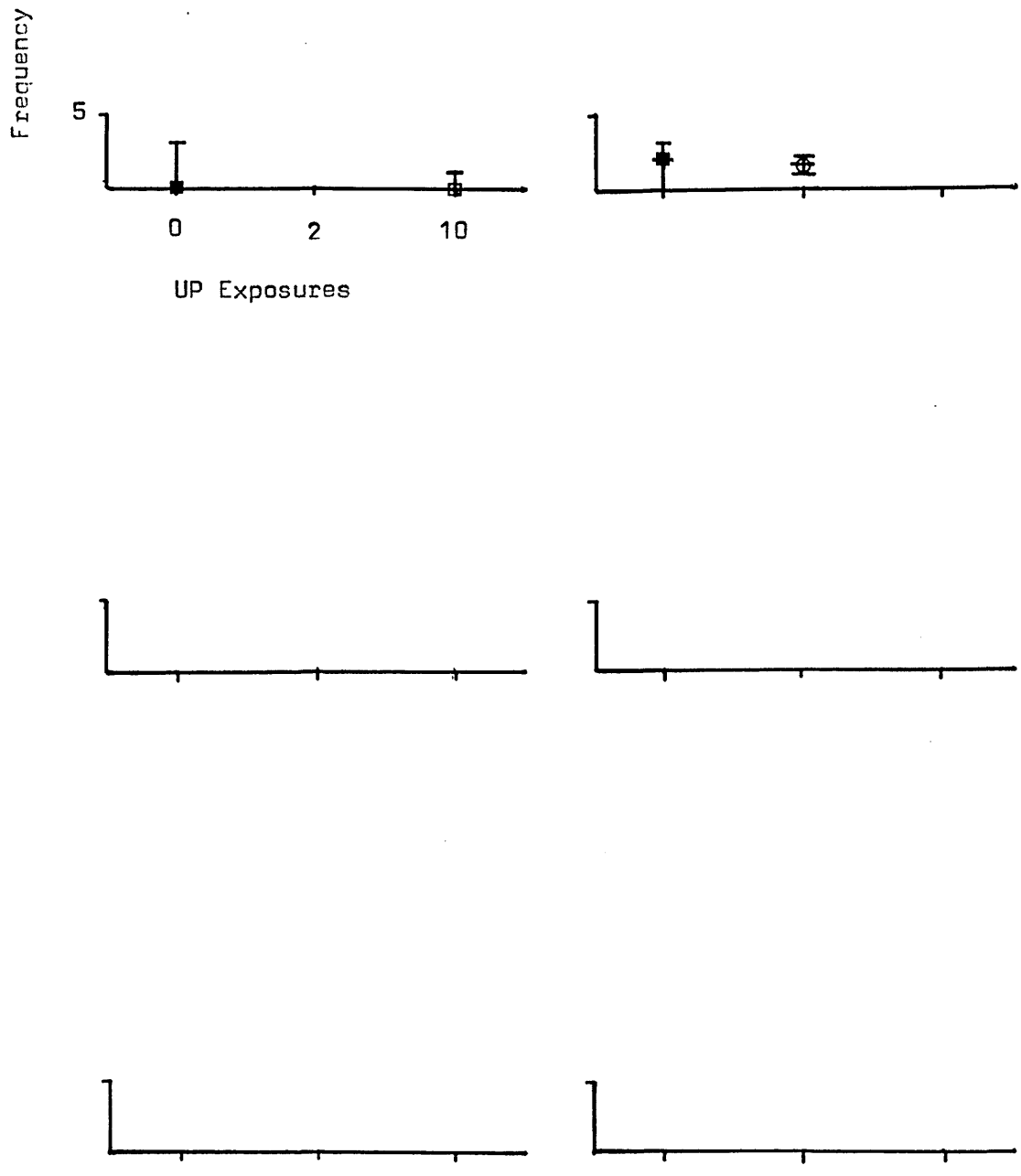

Figure 8.12 Retrieve
(Legend as for figure 8.1)

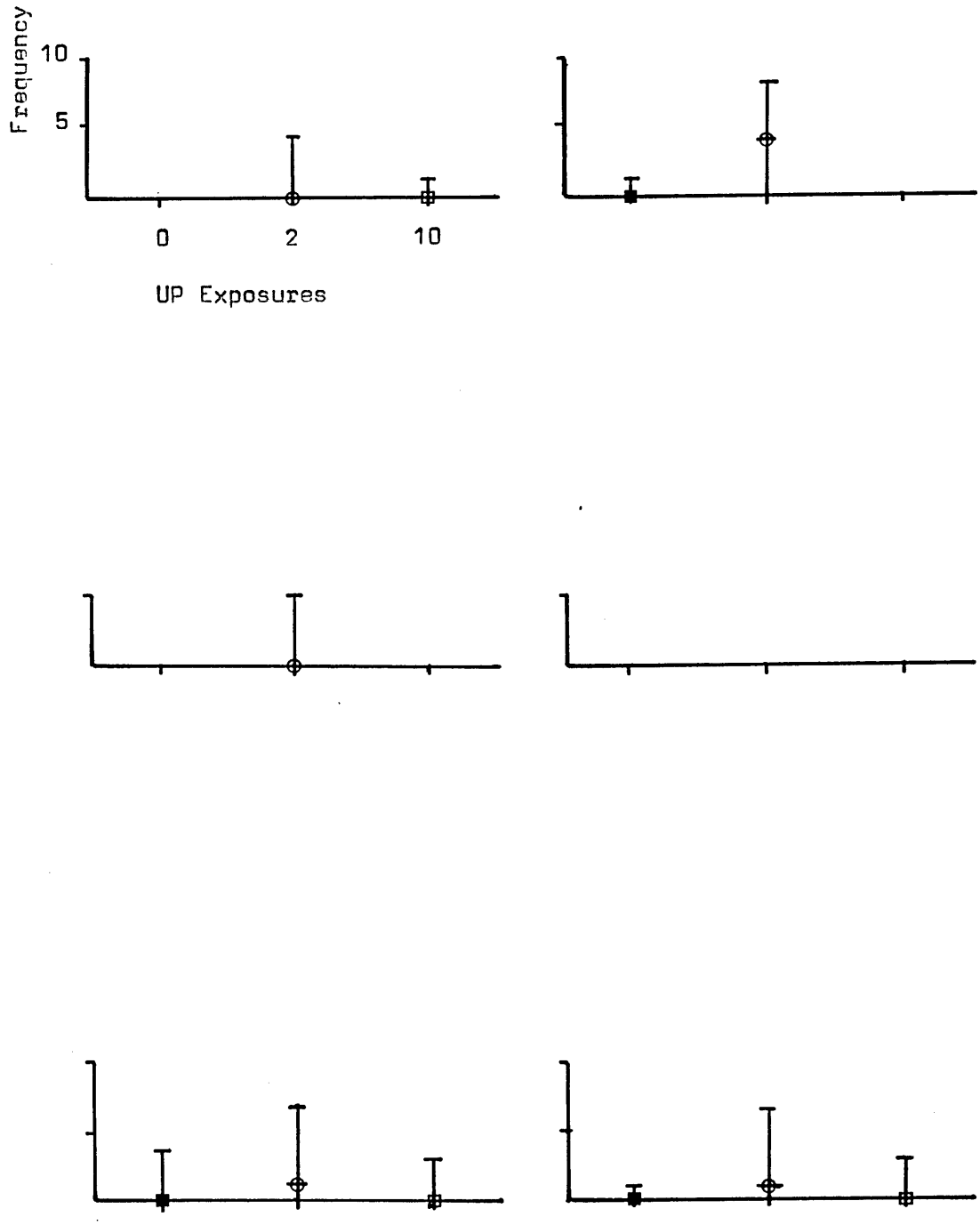

Figure 8.13 Gather

(Legend as for figure 8.1)

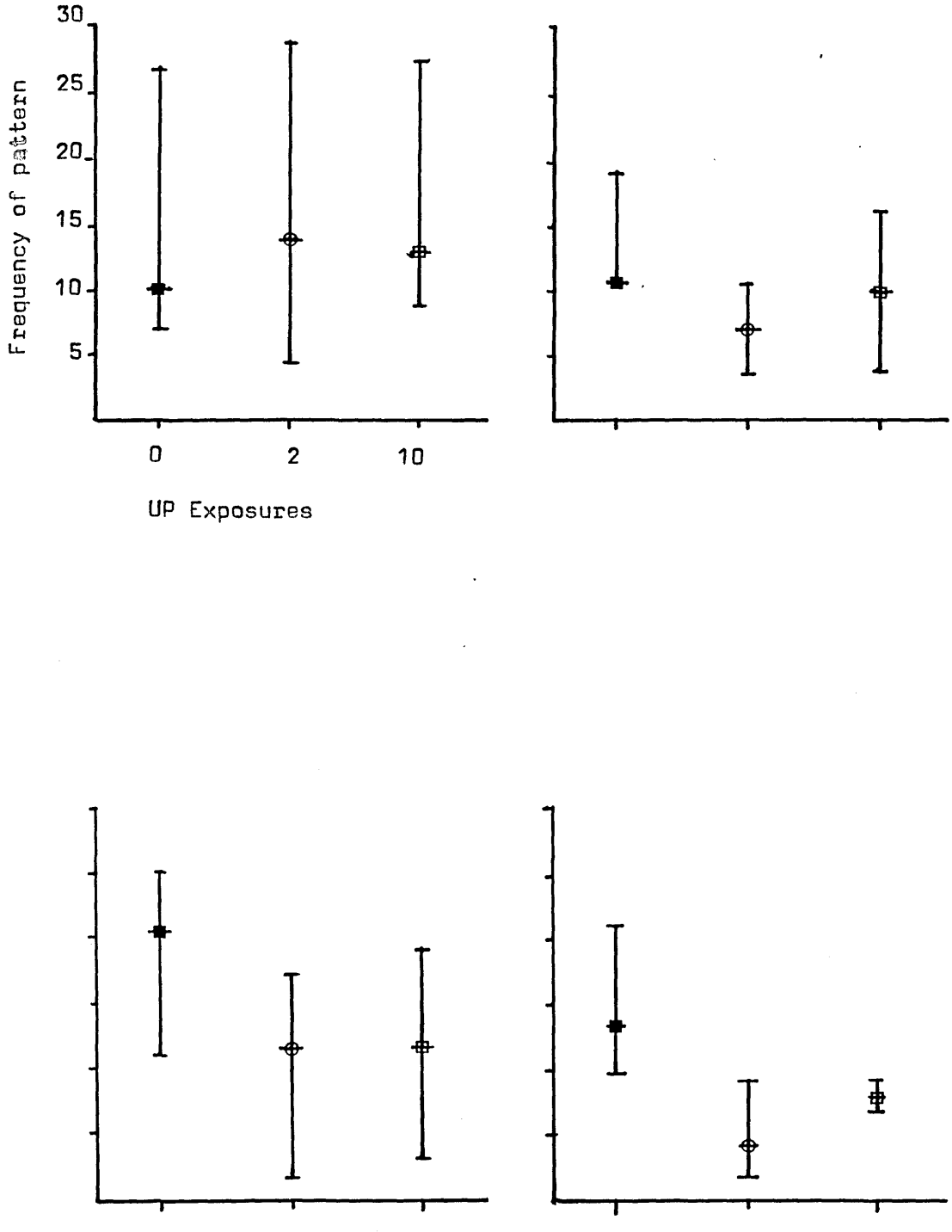

Figure 8.14 Total ≤2.5cm from pup
(Legend as for figure 8.1 except
top graphs = Group 1, bottom = Group 2)

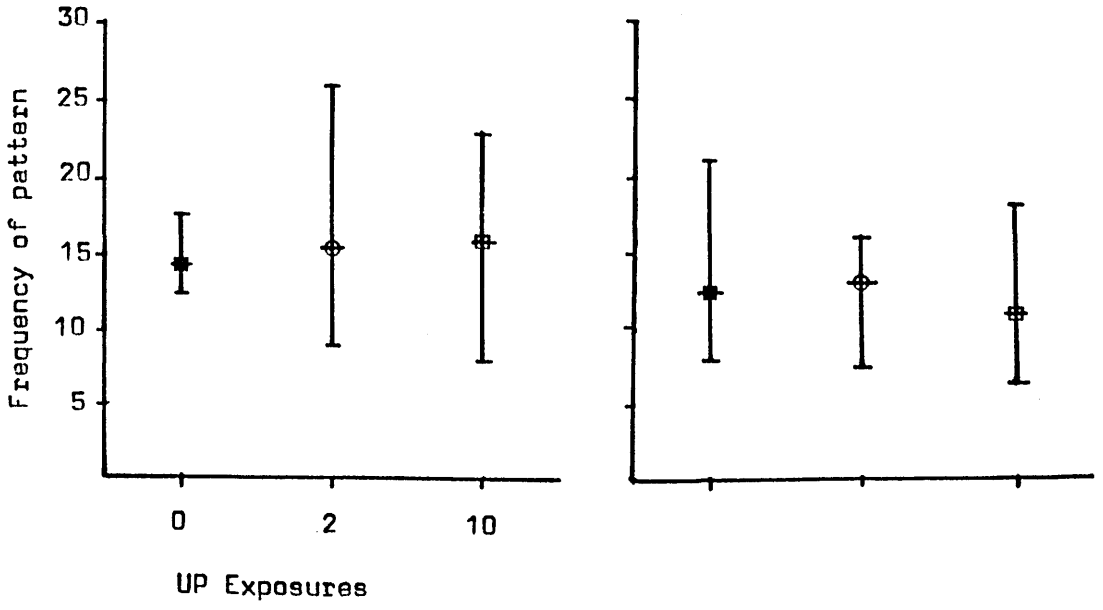

Figure 8.14 (contd) Total ⩽2.5cm from pup

(Legend as for figure 8.1 except left hand graph = Group 3 males, right hand = Group 3 males)

pup and nest-building (Fig. 8.11) from original to 10wk M UP exposures ($T = 0$, $p < 0.05$). Due to small sample size, statistical analysis was not possible for Group 1 males and females, Group 2 females and Group 3 females except for the comparison between original and 10wk M UP exposures.

8.3 iii Sex differences

No significant sex differences were seen in the percentages of aggressive responses in UP exposures in any group at any time interval (Table 8.2). But in general, twice as many females as males were aggressive on at least one occassion in a series of UP exposures (Table 8.3). Group 2 animals (Table 8.4) showed a significant sex difference in time spent burrowing at 2wk M UP exposures, with females showing more than males ($U = 2$, $p < 0.038$). Group 3 males (Table 8.5) showed significantly more pup-sniffing than females ($U = 7$. $p < 0.05$), and sat over the pups more than females did ($U = 6$, $p < 0.02$) at the 2wk M UP exposures. Females spent more time burrowing than males, both at the 2wk M UP exposures ($U = 7, p < 0.05$),and at the 10wk M UP exposures ($U = 9$, $p < 0.02$) (Tables 8.5 and 8.6). No other significant sex differences were seen in the frequencies of behaviour patterns. (Differences at original UP exposures are discussed in the relevant chapters).

Summary

1. Overall, males showed a decrese in aggression in the short term (2 weeks), with a slight increase in the long term (10 weeks).
2. Females showed an increase in aggression both in the short and the long term.
3. On the whole, males spent more time in parental behaviour

Table 8.4 Comparison of frequencies of behaviour patterns shown by Group 2 males and females at 2wk M UP exposures.

Pattern	Males*		Females*		p△
Inactive	1.0	(0.75-2.0)	1.0	(0.25-4.0)	
Groom self	1.0	(0.75-3.25)	2.0	(1.25-4.62)	
Scratch cage	3.0	(0-10.5)	3.8	(0.1-18.3)	
Sniff own cage	7.5	(4.75-8.5)	5.0	(5.0-9.5)	
Sniff pup	6.0	(4.25-9.25)	4.25	(2.38-5.0)	
Tear paper	1.0	(0-8.75)	1.0	(0.25-1.375)	
Gnaw own cage	2.5	(0-6.0)	0	(0-7.5)	
Burrow	0.5	(0-5.5)	13.0	(5.5-20.5)	< 0.038
Bury pup	0	(0-0)	0	(0-0)	
Sit over	0.5	(0-5.75)	0	(0-0)	
Sit over + NB	0	(0-0)	0	(0-0)	
Gather	0	(0-0)	0	(0-0)	
Total ≤ 2.5cm	11.5	(5.0-16.25)	4.25	(2.38-8.0)	

* Median of medians with interquartile values in parentheses.

△ Mann-Whitney U test.

Table 8.5 Comparison of frequencies of behaviour patterns shown by Group 3 males and females at 2wk M UP exposures.

Pattern	Males*		Females*		p△
Inactive	1.0	(0-2.5)	0	(0-0)	
Groom self	0	(0-1.13)	0.5	(0-1.0)	
Scratch cage	0.25	(0-2.0)	1.0	(0.5-2.0)	
Sniff own cage	14.25	(12.0-17.25)	12.0	(8.0-17.0)	
Sniff pup	6.5	(4.5-9.38)	3.0	(2.0-5.0)	< 0.05
Tear paper	0	(0-1.0)	1.0	(0.5-3.5)	
Gnaw own cage	0	(0-0.125)	0	(0-0)	
Burrow	2.5	(0.38-4.0)	9.0	(3.5-10.0)	< 0.05
Bury pup	0	(0-0)	0	(0-0)	
Sit over	2.5	(1.13-4.75)	0	(0-0.5)	< 0.02
Sit over + NB	0	(0-2.13)	0	(0-0)	
Gather	1.25	(0.75-4.25)	1.0	(0-4.25)	
Total ⩽ 2.5cm	15.5	(12.25-19.5)	13.0	(9.75-15.0)	

* Median of medians with interquartile values in parentheses.

△ Mann-Whitney U test.

Table 8.6 Comparison of frequencies of behaviour patterns shown by Group 3 males and females at 10wk M UP exposures.

Pattern	Males*		Females*		p△
Inactive	1.0	(0-3.0)	0	(0-1.5)	
Groom self	0.5	(0-1.5)	0	(0-0.5)	
Scratch cage	0	(0-0.75)	0	(0-0.5)	
Sniff own cage	14.0	(11.25-15.5)	15.0	(13.0-20.5)	
Sniff pup	5.0	(3.5-7.75)	4.5	(4.0-7.5)	
Tear paper	0	(0-1.0)	0	(0-1.0)	
Gnaw own cage	0	(0-0)	0	(0-0)	
Burrow	0	(0-4.0)	7.0	(4.0-19.0)	< 0.02
Bury pup	0	(0-0)	0	(0-0)	
Sit over	5.5	(0-13.0)	0	(0-2.5)	
Sit over + NB	1.0	(0-3.25)	0	(0-0.5)	
Gather	0	(0-2.5)	0	(0-0)	
Total ⩽ 2.5cm	16.0	(10.0-22.5)	11.0	(8.0-18.0)	

* Median of medians with interquartile values in parentheses.

△ Mann-Whitney U test.

patterns than females did.
4. In general, twice as many females as males were aggressive to pups.

8.4 Discussion

8.4 i Aggression

Studies of parent-offspring relationships (Elwood, 1977), with regard to the maintenance of non-aggressive responses to pups suggest that if a female was non-aggressive and fully parentally responsive at the original UP exposures in such work as that described above, then she would still be non-aggressive at the 2 wk M exposures, since this would naturally fall within the lactation period after gestation and parturition. In males it would be expected that once a non-aggressive response had been produced, this would always be maintained. It would be expected that by the 10 wk M exposures, females would have reverted to aggressive responses to pups, since pups would no longer be at all dependent on their parents. Again, males would be expected to retain any non-aggressive responses which had been produced earlier, since this is the case with fathers.

The method used for maintenance experiments with the group 1 animals allowed a brief exposure to protected pups prior to the UP M exposures, but from previous results, it seems unlikely that the 2 or 3 PP exposures would be enough to cause the animals to lose their aggression to pups. This is in fact supported by the results for the females in the group, since there was little difference in

the percentages of aggressive animals between original and 2 wk M exposures, but all of the females were aggressive on at least one of the 10 wkM exposures. These results are as would be expected. The sample size was too small to show up differences statistically, but it certainly seems that any loss of aggression produced by original exposures to pups is no longer evident 10 weeks after the effect was produced. Males showed a decrease in percentage animals showing aggression from the original to the 2 wk M exposures, which suggests the intermediate 3PP exposures may have had an effect on them, perhaps due to males advancing through the stages in the development of parental responsiveness more quickly than females do. The percentage of animals aggressive from 2 to 10 wk M exposures was virtually maintained. This is also the result which would have been expected in view of what is known of the maintenance of parental responsiveness in fathers.

In the maintenance exposures for group 2 animals, no PP exposures were given prior to the UP exposures. Results here were again as would be expected from what is known of the natural situation, that is, there was little difference seen in the percentage of animals showing aggression between original and 2wk M exposures. Males showed little or no difference between 2 and 10 wk M UP exposures, while females showed an expected increase in aggression.

In the group 3 maintenance exposures, males showed an increase in percentage animals responding aggressively from both original to 2 wk, and from 2 wk to 10 wk M UP exposures. Females

also showed this increased percentage of animals showing aggression. Since the main difference between the method in groups 1 and 2, and group 3 is that the pup was rubbed on the experimental adult's scent gland in Group 3 animals, it may be that this had an effect on the maintenance of the response produced, although this would not be expected, since it seems the familiar odour on the pup reduces aggression and/or promotes the development of parental responsiveness. (See Chapter 7 for further discussion). Another difference between the methods of maintenance exposures in groups 1 and 2, and group 3 was that many more UP exposures were actually carried out with the group 3 animals. This would have been expected to have reduced aggression. The implication from these results is that the way in which an animal is made to be parentally responsive determines whether or not the effect will be maintained. It is possible that the familiar odour on the pup only helps to overcome aggression to pups, and that the following development of parental responsiveness is not as permanent as when aggression is overcome without the familiar odour.

8.4 ii Parental patterns

As stated earlier, from studies of encounters between gerbil parents with strange pups, males here showing parental responsiveness at original UP exposures would be expected to retain this indefinitely. Females however, would be expected to lose it between the two maintenance testing intervals, that is 2 and 10 weeks after original UP exposures.

Times spent sniffing the pup decreased from original to 2wk M UP exposures in 5 of the 6 groups of animals (Groups 1 - 3, male and female), with this decrease being significant in Group 2 males. This supports earlier theories of the role of the odour of the pup in the development of parental behaviour. It also suggests that as with parents, parental responsiveness is maintained 2 weeks after it is produced, since here the pup is not being investigated as a novel stimulus would be if responsiveness had not been maintained. The level of pup sniffing from 2 to 10wk M UP exposures varies, with two groups of males showing a similar level, and one showing an increase. The two showing no change suggest parental responsiveness is maintained over time, perhaps with males recognizing the pup as such (that is a pup requiring parental care), without increased investigation. Two groups of females show an increase in their level of pup-sniffing from 2 to 10wk M UP exposures, suggesting the first stage in the process of the development of parental responsiveness is starting again, with an increase in investigative behaviours.

Burrowing shows little change in frequency over time with males. This pattern was earlier suggested as being due to disturbance from the pups. Results therefore suggest that males responding parentally towards pups due to exposure to pups continue to tolerate them, whereas the females' overall increase in burrowing suggests once more that they are disturbed by the presence of the pup in their cage.

Two groups of males showed an increase in amount of time spent sitting over the pup over time, with a significant difference between original and 2wk M UP exposures for Group 3 males. This suggests a cumulative effect of the stimulation from pups producing more parental behaviour with increased exposures to pups.

One group of females showed a slight increase in time spent sitting over the pup, although the others showed no such increase, with a decrease in Group 2. This suggests the level of parental responsiveness in females falls off due to lack of stimulation from the pups.

Another parental response shown by both males and females was 'sit over and nest-build'. Little of this behaviour was seen, but in Group 3 males a slight increase was seen from 2 to 10wk M UP exposures, wherease females showed no change. This is likely to be due to the same reasons as those discussed for sitting over the pup. Nest-building might have been expected to decline from original to 2wk M levels (as seen in Group 2 males and Group 3 females) since pups present with their parents at this age are capable of thermoregulation (McManus, 1971), and require less help in maintaining their body temperature. However, in this experiment, pup stimuli at the 2wk M UP exposures were provided by pups young enough to still be dependent on their parents (and the nest material) for warmth, so this may not account for these two specific behaviour patterns declining at these times.

Time spent by females 'sniffing own cage' in general showed little change from original to 2wk M UP exposures, then an increase from 2 to 10wk M UP exposures. This supports the idea that parental responsiveness produced during original UP exposures, and maintained at 2wk M UP exposures is lost, with the process beginning again when the females are once more exposed to pups. 'Sniffing own cage' may well be part of the investigation seen at the start of the development of parental responsiveness. Only one group of males showed this increase from 2 to 10wk M UP exposures, although Group 3 males showed a significant increase from original to 2wk M UP exposures. Since the method used in Group 3 involved the alteration of the pups' odour, this increase may well be due to the method used.

Total time spent near the pup tended to stay more or less the same, or decrease from original to 2wk M UP exposures in males and females, then stay the same from 2 to 10wk M UP exposures with males, but increase from 2 to 10 wk M UP exposures with females. This too supports the general idea of the process of the development of parental responsiveness beginning again in females after a certain time without stimulation from pups, that is this could be a renewed increase in investigatory behaviour patterns.

8.4 iii Sex differences

Sex differences in levels of aggression and in levels of parental patterns over time have already been partly discussed in sections 8.4 i and 8.4 ii.

Significant sex differences were seen in time spent burrowing in Group 2 animals at 2wk M UP exposures, and in Group 3 animals at both 2 and 10wk M UP exposures, with females showing more of the behaviour than males in all cases. As discussed earlier, this suggests females are 'disturbed' by the presence of the pup in their cage more than males are.

Group 3 animals at 2wk M UP exposures also showed significant sex differences in times spent sniffing the pup and sitting over the pup, with males showing more of both patterns than females. The greater pup sniffing by males is not in line with expected results, and may again be a direct result of altering the pup's odour. The greater time spent by males sitting over the pups suggests males are more parentally responsive to pups at this stage of the experiments than females are, adding support to the idea of parental responsiveness developing in stages, and more quickly in males than in females.

On the whole in all groups, males spent more time in the parental behaviour patterns than females did. This is probably due to the hypothesis raised earlier, that different stages occur in the development of parental responsiveness, and that these occur more quickly in males than in females.

With regard to the maintenance of the developed parental responsiveness the males' parental responsiveness is maintained, whereas the females' declines. This is a similar finding to that seen in the case of male and female parents when later presented

with strange pups (Elwood, 1977).

Several hypotheses discussed here are in agreement with other earlier work. Maternal responsiveness to gerbil pups has been maintained for longer than would normally be expected by replacing pups with those of a younger age (Elwood 1981). This same work showed that normal maintenance of maternal responsiveness was dependent on the presence of the young. Elwood and Ostermeyer (1984c) have also suggested that the maternal aggression of the female during her pregnancy causes the subordination of the male, thus inhibiting his aggression towards pups, since subordinate males (as determined in male-male encounters) show much less infanticide than dominant males. It is not clear why males never revert to attacking pups once this has been overcome.

From the present work, it seems that the change in parental responsiveness is brought about by the influence of cues from the pups, with these possibly producing hormonal changes in the adults. The mechanisms suppressing the aggression and then allowing maternal responsiveness to occur seem the same as those in the 'normal' situation of laboratory studies of parent-offspring encounters since the maintenance effect over time is similar to that in the normal situation.

It seems unlikely here that the males are being affected by maternal aggression in the females since the females are not pregnant and are therefore not likely to have the same effect on

the male as in the natural situation. It therefore seems valid to conclude that males are influenced in the same way as females, that is by cues from the pups. Since this is not possible in the natural situation, due to no pups being present prior to parturition, it is possible that two mechanisms exist to bring a male into a parental state, dependent on whether he is exposed to pups or his pregnant mate. Since the maintenance effect is the same in pup-exposed parentally responsive males with 'normal' smelling pups as it is in fathers, the two mechanisms seem to have the same end result, that is they produce parental responsiveness which is maintained indefinitely. Parental responsiveness produced by altering the odour of the pup seems only to allow a faster and earlier development of parental responsiveness, and not the maintenance effect seen in laboratory studies and other experiments described here.

CHAPTER NINE GENERAL DISCUSSION AND CONCLUSIONS

It soon became apparent from the preliminary experiments that male and female adult Mongolian gerbils could be induced to show parental responsiveness by exposure to pups. Another point noted early on in the work was that the development of parental responsiveness was a non-unitary response, that is it appeared to occur in several stages. The stages suggested as occurring from evidence gathered here were: 1. the loss of fear of pups and the following or perhaps independent loss of aggression towards pups; 2. an aversion to pups; 3. an increase in investigative behaviour patterns, especially those directed towards the pup; and 4. the appearance of parental patterns. Noirot (1969b) noted that naive adult male and female mice showed parental patterns in a serial order following pup exposures. Two serial orders were seen most frequently. These were 1. retrieval, licking of pup, nest-building, lactation position and 2. retrieval, nest-building, licking of pups, lactation position. The reason suggested for the difference between the two was that those showing the latter order had difficulty retrieving the pup, often dropping it. When the pup was dropped in this way, the adult frequently ran back to the nest carrying, for example, a wood shaving. This was often followed by scratching, and the pattern was recorded as nest-building. In the current experiments with gerbils, retrieving was always one of the last responses to be seen. Sniffing and licking of the pup were almost always the first patterns seen, with nest-building commonly occurring next. The lactation position and retrieving were more rarely shown, and usually occurred later even than nest-building.

The reason given by Noirot to possibly account for these particular serial orders was that ultrasonic distress calls were emitted by the pup when it was outside the nest. These calls would stimulate retrieving and nest-building in the adult. Rough handling of the pup by the naive adult would cause pups to emit calls which might inhibit adult aggression, causing the adult to drop the pup. The pup then resuming the original type of calling would promote more nest-building then searching for the pup. On finding the pup, and after retrieving it to the nest, olfactory cues might stimulate pup licking. Once again rough handling of the pup due to the adult being naive could stimulate calls from the pup, inhibiting the adult's response. When the adult rested, the pup might approach and go under the adult, initiating the lactation position in the adult.

The only pattern seen in a common position in the order of responses shown by mice and gerbils is the ultimate lactation position. Reasons for the differences, apart perhaps from the two species simply having different mechanisms involved in the induction of parental responsiveness, would include the rates of emission of and types of ultrasonic calls. Since calls here were not all identified as coming from the pup, they cannot be correlated with serial order. Further work might include a more detailed study of the role of infant vocalizations in the development of parental responses. Ideally, the source of vocalizations would be identified, thus enabling the detection of any correlations between pup calling and adult behaviour.

Differences in the ease of priming might also account for species differences in the order of responses shown. A difference in results found here and in similar experiments described by other authors was in the total exposure time required to produce a parental response in naive adults. The shortest latency to a non-aggressive response seen here in gerbils was a total time of 30 minutes, found for both male and female animals exposed to pups bearing the sebum from the adults' scent glands. The latency may even have been shorter since adults were never exposed to unprotected pups any earlier than after three 10 minute protected pup exposures. Previous experiments have shown a need for pup exposures of up to 15 days for rats (Rosenblatt, 1967), although mice had previously responded parentally in a few minutes (Beniest-Noirot, 1958). This implies gerbils can be induced to respond parentally to pups more quickly than rats. This had not previously been detected since naive gerbils normally attack pups on their first encounters (this was overcome here by presenting the adults with protected pups). Future investigations would attempt to clarify why familiar odours speed up the priming process, and also why parental behaviour induced in this way is not maintained as long as that induced by pups with unaltered odours.

Several other hypotheses apart from hormonal mechanisms and pup exposure have been suggested for either the inhibition of pup killing or the development of parental responsiveness in rodents and other animals. Keverne _et al_ have suggested vaginal-cervical stimulation during parturition is the important factor in the

induction of maternal bonding and maternal behaviour in sheep and rats (Keverne et al,1983; Yeo and Keverne, 1986). In sheep (Keverne et al, 1983) artificial vaginal-cervical stimulation after oestrogen and progesterone preparation produced full scale maternal behaviour in non-pregnant, multiparous ewes. Stimulation given to recently parturient ewes allowed bonding to and adoption of alien lambs to occur. This was taken as evidence that vaginal-cervical stimulation has a role to play in the onset of maternal behaviour in sheep. With rats (Yeo and Keverne, 1986), vaginal-cervical stimulation promoted maternal behaviour in multiparous non-pregnant females, but not in virgin females. This was taken as evidence that "sensory input from the vagina plays an important role in the immediate induction of maternal behaviour, but this effect is synergistic with that of hormones and experience". Clearly vaginal-cervical stimulation may influence maternal behaviour occurring in multiparous females, but so far no effect has been found for virgin females, and the above mechanisms can have no relevance to males.

Elwood and Ostermeyer (1984a) have investigated the role of the act of copulation in inhibiting infanticide in males following several suggestions of a reducing effect (Huck et al, 1982; vom Saal and Howard, 1982). However it still seems that copulation alone is not sufficient to inhibit infanticide, and that subsequent cohabitation with a pregnant female is necessary. This appears to be true both for mice and gerbils.

As mentioned earlier, the shortest latency to a non-aggressive response was achieved when pups had been rubbed on the experimental adult's scent gland, and therefore bore the adult's own odour. A shorter latency was also seen when the pup had been rubbed with the experimental adult's urine than when the pup had its odour unaltered. A recent paper (Singh, Brown and Roser, 1987) suggests that the antigens of the major histocompatibility complex (MHC), found as cell-surface glycoproteins on an individual's cells and characteristic of the individual, may have a function in the recognition of individuals in rodents. It is suggested that the immunological interpretation of the MHC is in fact not its only function, and that the system may also serve in identification of individuals by identification of MHC glycoproteins by the vomeronasal organ or olfactory detection of volatile components of the MHC molecules in the urine. These molecules which are thought to be expressed in urine are known as 'MHC identifiers'. This explanation could be used to account for the reduced latency to an aggressive response to a sebum or urine smeared pup (seen in the current experiments) if the animals in the colony were highly interbred, and therefore shared common genetically derived elements in their MHCs. In fact the animals used here were mainly from the one colony (see section 2.1), and although brother - sister matings were prevented, cousin - cousin pairings were likely to have occurred.

A further conclusion from current experiments was that not only could twice as many males as females be 'primed', but that priming occurred more quickly in males than in females. Rosenblatt

(1967) described a 'basic maternal responsiveness' in rats, which when supplemented by pup stimulation, resulted in parental behaviour. It is possible that both males and females have a basic 'parental' responsiveness requiring supplementing by other means (for example pup stimulation or gonadal hormones). The threshold of this may be lower in males than in females, accounting for the difference in 'priming rates' outlined above. Another explanation is that females may be more aggressive or fearful than males, and if indeed aggression or fear must be overcome prior to parental responsiveness developing, then indeed males would develop their responsiveness more quickly than females.

The final topic investigated by the current study was how induced parental responsiveness compared in permanence with the normal parental behaviour found in laboratory studies. In general results were the same for the two methods, that is that females lose their responsiveness after raising a litter or being induced to act parentally, whereas the response in males is permanent once seen. One exception to this however, was with the animals 'primed' by exposure to 'sebum-scented' pups, which all tended to lose their responsiveness even within two weeks. For some reason, it seems that this method of short-latency parental priming is also of short duration. This may even suggest that otherwise pup exposures have a memory effect, and that the stimulus is recognizable after sufficient exposure. An odour familiar to the adult appearing on the pup may effectively by-pass this memory effect, and therefore be no longer efficient within two weeks. If this memory effect is in operation, there may be sex-differences

in it which account for the differences found here between males and females.

As always, more research into the subject would possibly answer some of the questions so far left unanswered. Some suggestions have been made throughout this discussion, for example, more detailed studies of ultrasonic vocalizations and also short latency priming with pups bearing odours familiar to the adults. Further 'odour' experiments which would be interesting would include tests where adults were exposed to pups bearing the adult's partner's sebum, and separately, urine. Repeats of several of the odour experiments, but using anosmic adults, would be useful in confirming results or possibly detecting alternative explanations for the results found in current experiments.

A subject not investigated here at all, and certainly of interest is the hormone levels/changes occurring during pup-induced parental responsiveness. Several ovarian hormones, especially oestrogen, are thought to exert a facilitatory influence on maternal responsiveness in naive female rats (for example see Mayer and Rosenblatt, 1979) and several other rodent species. Other experiments have been carried out in which blood samples for hormone-level measurements were obtained by the use of intraatrial cannulas. A system similar to this could be used to make these measurements in further investigations. Several pituitary hormones, for example oxytocin and prolactin have also been suggested as having a role in the induction of parental behaviour, often working in conjunction with ovarian hormones

(for example, Pedersen and Prange, 1979). These are discussed in detail in Chapter One). These too could be measured in further investigations. The use of ovariectomized female and castrated male adults could also be beneficial in future studies, since these animals can have gonadal hormone levels controlled by the experimenter by injection. Another factor related to this and possibly affecting results but as yet not taken into consideration is the females' stage of oestrous.

Clearly the achieving of parental responsiveness by exposure to pups in Mongolian gerbils has filled a gap in rodent behaviour studies, and some of the means by which this can be achieved have been elucidated.

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ELSPETH C. HENRY. BEHAVIOURAL MECHANISMS UNDERLYING INFANT CARE IN MALE AND FEMALE MONGOLIAN GERBILS.

The induction of parental behaviour in naive animals has been studied in several species. Male and female adult Mongolian gerbils, Meriones unguiculatus were singly exposed to protected pups (PP exposures). Exposures lasted for ten minutes, and were carried out each day. Behaviour patterns shown by the adults were recorded, and their frequencies measured. After several PP exposures (3 - 18), adults were exposed to unprotected pups (UP exposures) in order to detect whether or not the normal aggressive response to pups was still present, or had been overcome and replaced by parental responsiveness. Preliminary experiments showed the aggressive response could be overcome in as little as five ten minute exposures. A variety of parental behaviour patterns were shown by some individuals, suggesting that stages may exist in the process under investigation. Following on from preliminary experiments, the effect of increasing the number of both PP and UP exposures was investigated. Increasing PP and UP exposures increased the percentage of animals responding non-aggressively towards pups. However no increase was seen in the range of parental responses shown. Again, results suggested the development of the parental response was a non-unitary process occurring in stages: first the overcoming of fear of pups or aggression towards pups; second, investigation of the pup; third, the development of parental responsiveness.

The role of olfactory and auditory cues from the pups were next investigated. If a pup bore the scent gland sebum of the experimental adult, aggression was overcome more quickly than before. Also, more parental behavior patterns were shown. If the pup bore the experimental adult's urine, aggression was overcome more quickly than in preliminary experiments, but not as quickly as when the pups bore the adult's sebum.

No correlation was found between the rate of ultrasonic calling and the rate of the induction of parental responsiveness. This was thought to be an artefact of the recording procedure, since the source of individual calls was not identified, and the frequency of calls could therefore have been increased due to adults calling.

Parental responsiveness appeared to be maintained 2 weeks after its induction, but not 10 weeks after induction. An exception to this was the animals exposed to pups smeared with the experimental adults sebum, who did not appear to maintain responsiveness even up to 2 weeks after induction.

Overall twice as many males as females were able to be induced to show parental responsiveness. Males overcame their aggression to pups, and showed parental responsiveness more quickly than females did.

Further work arising from the present studies would include a more detailed study of both the influence of ultrasonic calling by pups on the development of parental responsiveness and the quicker development of parental responsiveness found when pups bore an odour familiar to the adults.